

University of Nevada, Reno

**Ecological Genetics of the Mojave Desert Tortoise**

A dissertation submitted in partial fulfillment of the  
requirements for the degree of Doctor of Philosophy in  
Ecology, Evolution, and Conservation Biology

by

Bridgette E. Hagerty

Dr. C. Richard Tracy, Dissertation Advisor

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## THE GRADUATE SCHOOL

We recommend that the dissertation  
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**BRIDGETTE E. HAGERTY**

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C. Richard Tracy, Ph. D., Advisor

Mary Peacock, Ph. D., Committee Member

Guy Hoelzer, Ph.D., Committee Member

Lynn Zimmerman, Ph.D., Committee Member

Leah Wilds, Ph. D., Graduate School Representative

Marsha H. Read, Ph. D., Associate Dean, Graduate School

December, 2008

## ABSTRACT

Understanding the population structure within a species, and understanding the processes shaping those patterns are important for basic and applied ecology. Here, we used genetic data and theory about population genetics to investigate the spatial structure of the desert tortoise (*Gopherus agassizii*) in the Mojave Desert, and used genetic analyses to induce the potential factors that created population structure. The desert tortoise is listed as threatened under the U.S. Endangered Species Act of 1973 in the northern extent of its range, which occurs north and west of the Colorado River. This distinct population segment has experienced severe population declines mainly resulting increased human impacts in the southwestern United States. The life history traits (i.e., long life span) and cryptic behavior of the desert tortoise make extensive field studies on population dynamics difficult. Thus, it was necessary to use highly variable, neutral genetic markers and analyses based in population genetic theory to make inferences about the population ecology of this species. The goals of this research were to identify genetic population boundaries, assess levels of gene flow among subpopulations, and determine the biological and physical landscape features that influence movement of individuals through habitat in the Mojave Desert. Additionally, we provide several recommendations to revise conservation strategies for the Mojave desert tortoise.

Despite discovering low levels of genetic differentiation among tortoises across the geographic range, we were able to detect hierarchical structuring within the population. Three basal groups were identified that correspond to the mitochondrial DNA haplotypes identified by others two decades previously. Within these three basal groups,

we detected seven subpopulations that loosely align with major geographic features. Geographic distance among subpopulations was a strong determinant of population structure, which suggests that localized dispersal is occurring across the geographic range. To investigate additional factors influencing movement of desert tortoises, we used a landscape genetics approach. We tested multiple hypotheses to determine which landscape features best correlate to patterns of gene flow. Landscape-genetic models supported the hypothesis that topographical features such as mountain ranges explain additional patterns in genetic substructure beyond a simple isolation-by-distance model.

The long generation time of desert tortoises contributes to a time lag in the genetic patterns identified by our analyses. Therefore, the inferred patterns of gene flow did not include any potential disruption from human activities such as habitat modification due to urbanization and the development of human infrastructure such as major highways. This unique situation allowed me to make conservation recommendations based on a genetic snapshot of historic population processes. Our main recommendations pertain to revising conservation units, maintaining landscape connectivity, and improving translocation of individuals. We provide suggestions for adjusting the boundaries of recovery units based upon genetic data as well as differences in ecology and behavior of desert tortoises that occur across environmental gradients in the Mojave Desert. We identify habitat corridors that have been historically important for connectivity among subpopulations, and we proffer potential management actions to maintain connectivity. Finally, we describe how genetic data can provide additional guidance for where individuals should be translocated.

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The research described in my dissertation would not have been successful without the support of many agencies and individuals. The impetus for this research was the Desert Tortoise Recovery Plan Assessment, which described a need for a comprehensive population genetic assessment of the Mojave desert tortoise. I would like to thank the members of the assessment committee (DTRPAC), C. Richard Tracy, Earl McCoy, Dave Morafka, Dave Delehanty, Michael Reed, Ken Nussear, Roy Averill-Murray, Bill Boarman, Jill Heaton, and Phil Medica for the discussions and moral support that lead me to pursue this research.

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## TABLE OF CONTENTS

ABSTRACT .....	i
ACKNOWLEDGEMENTS .....	iii
LIST OF TABLES .....	x
Chapter 1 .....	x
Chapter 2 .....	x
Chapter 3 .....	xi
Appendix A .....	xi
Appendix B .....	xii
LIST OF FIGURES .....	xiii
Chapter 1 .....	xiii
Chapter 2 .....	xiii
Chapter 3 .....	xiv
Appendix B .....	xiv
CHAPTER 1. DEFINING POPULATION BOUNDARIES FOR THE MOJAVE DESERT TORTOISE .....	
ABSTRACT .....	1
INTRODUCTION .....	2
STUDY AREA .....	7
METHODS .....	10
Sampling Design .....	10
Sample Collection, DNA Isolation, and Genotyping .....	12
Descriptive Population Genetic Analyses .....	13
Identifying Subpopulations .....	14
Statistics for Inferred Subpopulations: .....	19
RESULTS .....	20
Descriptive Population Genetic Analyses .....	20
Identifying Subpopulations .....	21
Statistics for the Inferred Subpopulations and Sampling Locations .....	25
DISCUSSION .....	27
Identifying Meaningful Genotype Clusters for the Mojave Desert Tortoise .....	27
Limitations to Identifying Desert Tortoise Subpopulations .....	33
Comparison to Other North American Tortoises .....	38
Recommendations for Conservation Practices .....	43
CONCLUSIONS .....	52
ACKNOWLEDGEMENTS .....	53
LITERATURE CITED .....	54
TABLES .....	69
FIGURE LEGENDS .....	77
FIGURES .....	79
CHAPTER 2. MORE THAN ISOLATION BY DISTANCE: A LANDSCAPE GENETIC APPROACH TO IDENTIFYING THE POPULATION STRUCTURE OF THE MOJAVE DESERT TORTOISE .....	
ABSTRACT .....	87

INTRODUCTION .....	88
MATERIALS AND METHODS .....	94
Desert Tortoise Genotyping .....	94
Straight-line Geographic Distance .....	96
Habitat Models for the Mojave Desert Tortoise.....	97
Two Models of Landscape Connectivity .....	99
Model Comparison .....	103
RESULTS .....	104
Relationship between Landscape Heterogeneity and Genetic Distance: Isolation-by-distance .....	104
Relationship between Landscape Heterogeneity and Genetic Distance: Least Cost Path ..	104
Relationship between Landscape Heterogeneity and Genetic Distance: Isolation-by-Resistance .....	107
DISCUSSION .....	109
Model Comparisons .....	110
Spatial and Temporal Influences on Long-term Movements in Desert Tortoises .....	114
Conservation Implications of Landscape Connectivity .....	116
Limitations of the Methods .....	119
CONCLUSIONS .....	124
ACKNOWLEDGEMENTS.....	125
LITERATURE CITED .....	125
TABLES .....	141
FIGURE LEGENDS.....	146
FIGURES.....	148
 CHAPTER 3. NEW INSIGHTS INTO CONSERVATION OF THE DESERT TORTOISE: IMPORTANCE OF GENETIC ANALYSES .....	 152
ABSTRACT .....	152
OVERVIEW .....	153
DESIGNATING CONSERVATION UNITS .....	155
TRANSLOCATION AS A MANAGEMENT TOOL .....	165
MAINTAINING CONNECTIVITY AMONG POPULATIONS.....	173
Habitat Fragmentation and Bottlenecking.....	177
Habitat Features Influencing Tortoise Movements .....	179
CONCLUSIONS .....	182
LITERATURE CITED .....	183
TABLES .....	197
FIGURE LEGENDS.....	202
FIGURES.....	203
 APPENDIX A. PRIMER NOTE FOR MICROSATELLITE MARKERS FOR THE DESERT TORTOISE .....	 206
 APPENDIX B: SUPPLEMENTAL METHODS AND RESULTS FOR CHAPTER 1	215
Supplemental Description of Population Genetic Analyses .....	215
Supplemental Tables and Figures from Chapter 1 .....	219
Supplemental Tables .....	219
Supplemental Figures .....	223

## LIST OF TABLES

### Chapter 1

Table 1	Sampling locations based on geography (including the state and abbreviation for the site), the number of individuals from each location, and how samples were collected .....	69
Table 2	Mean gene diversity ( $\pm 1$ standard deviation), mean allelic richness ( $\pm 1$ standard deviation), and $F_{IS}$ (significant values after Bonferroni correction of $P < 0.0001$ are in bold) for each sampling location .....	70
Table 3	Mean $\ln P(D)$ ( $\pm 1$ standard deviation) and the second order rate of change calculations for $\Delta K$ when $K$ was fixed to $K = 1$ through $K = 10$ in STRUCTURE .....	71
Table 4	Mean $\ln P(D)$ and $\Delta K$ for each of the three basal clusters in STRUCTURE. These additional analyses were used to detect hierarchical clustering within the Mojave population of the desert tortoise .....	72
Table 5	Log of the posterior density of the model for 10 independent runs of GENELAND .....	73
Table 6	Analysis of molecular variance for 3 genotype clusters as determined via STRUCTURE .....	74
Table 7	Pair-wise $F_{ST}$ values for the 9 inferred genotype clusters .....	75
Table 8	Number of assignments to one of the nine inferred genotype clusters .....	76

### Chapter 2

Table 1	Sampling locations based on geography (including the state and abbreviation for the site), and the number of individuals from each location .....	141
Table 2	Mantel correlations between pair-wise population Euclidean distance, resistance distance (IBR), or least-cost distance (cost or length) and genetic distance ( $F_{ST}/(1-F_{ST})$ , $D_S$ , $D_{LR}$ ) .....	142

Table 3	Mantel correlation between pair-wise individual Euclidean distance, resistance distance (IBR), or least-cost distance (cost or length) and genetic distance ( $D_{PS}$ or $a_r$ ) .....	143
---------	---	-----

Table 4	Partial Mantel correlations between pair-wise population resistance distance (IBR), or least-cost distance (cost or length) and genetic distance ( $F_{ST}/(1-F_{ST})$ , $D_S$ , $D_{LR}$ ), while accounting for geographic distance .....	144
---------	---	-----

Table 5	Partial Mantel correlations between pair-wise individual resistance distance (IBR), or least-cost distance (cost or length) and genetic distance ( $D_{PS}$ or $a_r$ ), while accounting for Euclidean distance .....	145
---------	---	-----

### Chapter 3

Table 1	Assignment of resident individuals in the Large Scale Translocation Site near Jean, NV to one of the nine genotype clusters identified using individual-based Bayesian assignment tests .....	197
---------	---	-----

Table 2	Assignment of translocated individuals in the Large Scale Translocation Site near Jean, NV to one of the nine genotype clusters identified using individual-based Bayesian assignment tests .....	198
---------	---	-----

Table 3	Private alleles found in nine subpopulations of the Mojave desert tortoise .....	199
---------	--	-----

Table 4	Detection of 34 first generation migrants of the Mojave desert tortoise .....	200
---------	---	-----

Table 5	Tests for population bottlenecks (BOTTLENECK and M-RATIO) in 25 sampling locations for the desert tortoise .....	201
---------	--	-----

### Appendix A

Table 1.	Desert tortoise microsatellite loci ( <i>Gopherus agassizii</i> (GOA)) combined in 4 multiplex PCR primer sets and one single PCR .....	213
----------	---	-----

Table 2.	Summary per locus across three populations of the desert tortoise in the Mojave Desert, USA.....	214
----------	--	-----

## Appendix B

Table 1. Mean proportional membership of each desert tortoise sampling location to nine genotype clusters as identified by STRUCTURE .....	219
Table 2. Analysis of molecular variance for 9 genotype clusters as determined via STRUCTURE .....	220
Table 3. Analysis of molecular variance for 4 genotype clusters as determined via GENELAND .....	220
Table 4. $F_{IS}$ values for 25 sampling locations of the desert tortoise across 20 microsatellites .....	221
Table 5. Pair-wise $F_{ST}$ values for all sampling locations of the desert tortoise (below diagonal) and corresponding pair-wise geographic distances (km; above diagonal) ...	222

## LIST OF FIGURES

### Chapter 1

Figure 1	Map of subpopulations for the Mojave population of the desert tortoise .....	79
Figure 2	Results from Program STRUCTURE using 20 microsatellites and 748 individuals from 25 sampling locations .....	80
Figure 3	Nine genotype clusters identified with Program STRUCTURE .....	82
Figure 4	Mean proportional membership ( $\pm 1$ standard deviation) to 6 genotype clusters identified using STRUCTURE when each sampling location has $n \leq 30$ .....	83
Figure 5	Representative bar plots for hierarchical structuring with a reduced data set. (a) Las Vegas Cluster, and (b) California cluster .....	84
Figure 6	Map of posterior probability of membership to four genotype clusters identified using GENELAND .....	85
Figure 7	Isolation by distance in populations of the desert tortoise across the Mojave Desert .....	86

### Chapter 2

Figure 1	Map of Mojave desert tortoises sampled for landscape genetics .....	148
Figure 2	Distribution of desert tortoise habitat in the Mojave desert predicted using the GRASP model in Program R .....	149
Figure 3	Cumulative least cost paths across 25 pair-wise population comparisons for different landscape variables (A) biological, (B) physical, (C) combined, and (D) binary.....	150
Figure 4	Cumulative current maps between pairs of populations from the isolation-by-resistance models using different landscape variables A) biological, (B) physical, (C) combined, and (D) binary .....	151

### Chapter 3

Figure 1	Map of the recovery units delineated in the 1994 Recovery Plan for the Mojave population of the desert tortoise .....	203
Figure 2	Map of subpopulations identified for the Mojave desert tortoise .....	204
Figure 3	Proposed revisions to conservation units for the Mojave desert tortoise .....	205

### Appendix B

Figures 1. Genotype clusters identified within the Northern Mojave cluster using hierarchical analyses in Program STRUCTURE .....	224
Figure 2. Genotype clusters identified within the Las Vegas cluster using hierarchical analyses in Program STRUCTURE .....	225
Figure 3. Genotype clusters identified within the California cluster using hierarchical analyses in Program STRUCTURE .....	226

## **CHAPTER 1. DEFINING POPULATION BOUNDARIES FOR THE MOJAVE DESERT TORTOISE**

### **ABSTRACT**

The Mojave desert tortoise is listed as a threatened species under the U.S. Endangered Species Act. It has a large geographic range, long generation time, and is cryptic because it spends a majority of its life underground in burrow. We used hyper-variable microsatellite markers to identify population structure, movements, and biological boundaries for subpopulations of the Mojave desert tortoise. Despite discovering low levels of population differentiation across the range, we were able to detect hierarchical structuring of the population using Bayesian assignment tests. Three basal groups were identified that correspond to the mitochondrial DNA haplotypes identified two decades previously. Additional structure was evident within each basal unit, and they were loosely concordant with major geographic barriers. Our conclusions about these three basal groups, and the substructure within those groups, required sampling tortoises from the entire geographic range, and sampling tortoises uniformly across the entire range in order to adhere to restrictions imposed by individual-based population genetic analyses. Another recent study differently concluded more fine-scale structure of desert tortoise subpopulations, but the investigators did not sample all parts of the species range, nor did they sample uniformly. Thus, their conclusions of greater genetic structure are based upon sampling for traditional population genetic analyses. Our conclusions about population structure translate into new recommendations for altering

the boundaries for recovery units for the Mojave desert tortoise. That translation indicates that approximately the same number of recovery units as prescribed in the Recovery Plan of 1994, but with very different boundaries, especially in Nevada.

## **INTRODUCTION**

Understanding population structure within a species, and understanding the microevolutionary processes shaping those patterns are important for basic (e.g., understanding population dynamics) and applied ecology (e.g., delineating conservation units). Natural populations are the products of a complicated mixture of historical and present demographic processes, which result in a cumulative genetic signature. These processes are intimately tied to geographic, landscape, and habitat features. Because of these processes, the underlying genetic population structure can provide an indication of how individuals move today and how they have moved in the past across a varied landscape. Additionally, genetic population structure can provide an indirect assessment of physical, ecological, and biological factors influencing movement (Manel et al. 2003, Waples and Gaggiotti 2006, Storfer et al. 2007). A rich literature in population genetic theory indicates that gene flow can be a powerful homogenizing force in evolution, constantly reducing genetic differentiation (though potentially enhancing polymorphism) in the face of mutation, selection, and genetic drift (Wright 1931, Lowe et al. 2004). Ultimately, gene flow causes mixing among populations, which opposes external factors that create divergence.

Delineating population boundaries can be complex for at least two reasons: natural borders can be unapparent, and some physical borders may not correspond with biological differentiation among populations. In many cases, population structure can be cryptic because a species' range is large and continuous without definitive geographic boundaries, and genetic differentiation may be influenced by unknown factors. The number of species with cryptic population structure is increasing in the published literature, particularly within highly mobile carnivores (e.g., Rueness et al. 2003, McRae et al. 2005, Pilot et al. 2006). Geographic distance often explains a portion of the spatial distribution of genetic variation among populations, especially in the absence of known barriers (Slatkin 1993). In these cases, levels of genetic differentiation on average are inversely related to the geographic proximity of populations (Wright 1943, Slatkin 1993). The influence of isolation-by-distance is mainly dependent on the dispersal ability of a species (Epperson 2003). Moreover, geographic, ecological, and behavioral barriers can influence gene flow beyond the simple process of isolation-by-distance (Lowe et al. 2004, Storfer et al. 2007). Typically, these unseen barriers complicate delineating population boundaries.

Relatively new population-level analyses using genetic data (e.g. Bayesian assignment tests; Pritchard et al. 2000, Manel et al. 2005) improve our ability to delineate genetic populations. These assignment methods rely on a Bayesian framework (Beaumont and Rannala 2004) to ascertain the membership of individuals to a population(s) using only multilocus genotypes (Manel et al. 2005). Most of these models use the assumptions of populations mating at random (i.e., at Hardy-Weinberg equilibrium and linkage equilibrium; Pritchard et al. 2000) to identify genetic

populations. Bayesian clustering methods provide a useful method for identifying structure in populations, especially those populations with distinct boundaries and high levels of differentiation (Pritchard et al. 2000, Evanno et al. 2005, Pritchard et al. 2007, Waples and Gaggiotti 2006).

A majority of population genetic analyses operate under the assumptions of the Wright-Fisher island model (Wright 1931). The island model assumes equal-sized populations with equal rates of gene flow among all pairs of populations. This model also assumes idealized populations (infinite and constant size, migration-drift equilibrium) and does not consider space implicitly or explicitly, which is unrealistic for most natural populations (Whitlock and McCauley 1999). Models of population structure that have a spatial component are likely to be more realistic, even if they are a simplification of actual structure. For example, the stepping stone model (Kimura 1953, Kimura and Weiss 1964) implicitly considers space by allowing movement only between adjacent discrete populations. The genetic signature of a population under the stepping stone model of population structure may not be distinguishable from Wright's isolation-by-distance model because distinct patches may not be identifiable with the genetic data collected.

Actual populations are neither truly panmictic nor completely isolated in most cases, and maintain varying levels of gene flow (Waples and Gaggiotti 2006). Bayesian methods can identify structure within populations that have dispersal patterns differing from a simple island or stepping stone model, such as models that include multiple levels of structure (i.e., hierarchy; Evanno et al. 2005). These methods also perform well when levels of differentiation in simulated populations are fairly low ( $F_{ST} = 0.02 - 0.03$ ; Latch et al. 2007). Further, models that explicitly use spatial data as prior information (e.g.,

GENELAND, Guillot et al. 2005a) have the potential to improve the ability to distinguish among populations with varying levels of differentiation (Guillot et al. 2005a, Coulon et al. 2006). Due to the potential intricacies of interpreting results from Bayesian methods, multiple analytical methods should be combined to infer the biology of gene flow (Rowe and Beebee 2007).

In the research reported here, we address complexities in identifying population boundaries, and the associated conservation implications, in the widely-distributed, threatened Mojave desert tortoise (*Gopherus agassizii*). The desert tortoise is distributed in the deserts of the southwestern United States and northwestern Mexico. The Mojave desert tortoise occupies both the Sonoran and Sinaloan Deserts, south and east of the Colorado River, and the Mojave and Colorado Deserts, north and west of the Colorado River (Germano et al. 1994). Only the Mojave population of the desert tortoise is listed as threatened under the U.S. Endangered Species Act of 1973. Within the range of the Mojave desert tortoise, habitat is extremely diverse, but relatively continuous from southwestern Utah to southwestern California. Pronounced population declines have been associated with several threats to population persistence, mainly attributed to increased human impacts within and on the Mojave Desert (USFWS 1994, Lovich and Bainbridge 1999, Edwards et al. 2004, Tracy et al. 2004). We will continue to use the naming convention of the Mojave population to include the region north and west of the Colorado River that we targeted for population genetic analysis. We will refer to all regions within the Mojave population as subpopulations because the level of divergence among groups within this region is unclear. We will refer to sampling locations as the geographic regions where genetic samples were collected.

As a long-lived species (generation time is 10-25 years) that is cryptic because it spends a majority of its life underground in burrow, the desert tortoise is an ideal candidate to infer population processes from genetic data. Relatively little is known about population dynamics or dispersal patterns for this species, which can be partially attributed to its low population densities and cryptic behavior. Desert tortoises, particularly hatchlings and juveniles, spend the majority of their time in retreats below ground (Nagy and Medica 1986, Morafka 1994, Hillard 1996, Wilson and Morafka 1999, Tracy et al. 2004). These characteristics make the collection of field data to make inferences about population size and dispersal more complicated, and population research on such a long-lived species generally requires long-term studies, which are logistically complicated (USFWS 1994). At the very least, population genetic data can provide some insight into population dynamics not generally possible from field studies.

Inferring population boundaries and translating those inferences into conservation planning can profoundly influence how management is implemented for such a species. The desert tortoise has a wide distribution, and its natural geographic distribution largely differs from political boundaries. The Mojave desert tortoise's range traverses four states (Utah, Arizona, Nevada, and California), and that range is currently divided into six recovery units (a management unit associated with a species' recovery plan; USFWS 1994). Preserving genetic and ecological diversity among populations continues to be a primary objective in desert tortoise conservation. The original recovery units reflected the best available scientific data at the time of listing (USFWS 1994), and they were delineated to preserve considerable variation in morphology (Weinstein and Berry 1987), ecology (Germano et al. 1994), and genetics (Lamb et al. 1989, Rainboth et al. 1989,

Lamb and Lydehard 1994). However, new data offer the opportunity to evaluate and revise those recovery units.

The main objective of our research is to characterize the population structure of the Mojave desert tortoise, using highly variable, nuclear, microsatellite genetic markers. Additionally, our goals are to evaluate the original 1994 Recovery Units, to make recommendations for potential revisions of their boundaries, and to compare recommendations generated here to those outlined in another recent study for this species (Murphy et al. 2007). We compared and contrasted individual-based methods for identifying genetic populations, which do not a priori require subjective groupings. Specifically, we inferred population structure in the Mojave desert tortoise using a genetic assignment approach which uses genotype data and a Bayesian statistical framework to delineate distinct breeding groups and infer gene flow. Additionally, we addressed the impacts and importance of sampling design for population genetic studies, and how sampling schemes can limit inferences made from these genetic markers.

## **STUDY AREA**

The Mojave desert tortoise is distributed within the Mojave and Colorado Deserts in California, southern Nevada, the southwest corner of Utah, and the northwest corner of Arizona (Fig 1). The Mojave and Colorado deserts ( $> 130,000 \text{ km}^2$ ) are heterogeneous in climate, geology, and topography (Berry et al. 2006), and vegetational associations (Rowlands et al. 1982). The range of geography and physiognomy of the desert tortoise distribution includes the lower reaches of the Colorado Plateau in Utah to physiographic

Great Basin in Southern Nevada and California. Each physiographic area has distinctive landforms and geological structure. A majority of the tortoise's distribution is encompassed by the larger Basin and Range Province within the Great Basin (Hunt 1974, Trimble 1989). Although plains and alluvial fans cover 65% of the Mojave Desert, mountain ranges, such as the Spring Mountains (3,652 m) and the Providence Mountains (2,148 m), provide commanding relief (Rowlands et al. 1982). Variation in elevation, slope, and soil type may be extremely important for habitat selection of this species (Andersen et al. 2000).

Abundance and seasonality of precipitation within the Mojave Desert is highly variable within and among years, but there is a consistent pattern of variation along a west-east gradient (Rowlands et al. 1982). Winter precipitation dominates in the Western Mojave, with greater than 75% of precipitation occurring between November and March, and less than 10% of precipitation occurring during the summer months of June - August (Germano et al. 1994). The percentage of summer and fall rainfall increases dramatically in the Eastern Mojave Desert (Germano et al. 1994). The phenology of annual vegetation, and the composition of the grasses and forbs is related to these differences. The majority of annual plants in the Western Mojave germinate during Fall and Winter months. Rainfall becomes more predictable in the southern portion of the Colorado Desert, which receives monsoonal precipitation typical of the Sonoran Desert (Burk 1977). Temperatures vary along a north-south gradient with the number of days below freezing, varying with both latitude and elevation. The number of freezing days decreases along a transect from southwestern Utah to the southern tip of the Colorado Desert in California (USFWS 1994).

Five major biotic regions occur in the Mojave Desert (Rowlands et al. 1982), and three regions occur in the Colorado Desert (USFWS 1994; Rowlands unpublished data). Vegetation is different in the Mojave and the Colorado Deserts. While many plant species overlap between these two deserts, the Colorado Desert contains some arboreal species that are sensitive to freezing (Burk 1977, Lovich and Bainbridge 1999). In many regions of the Mojave, creosote bush scrub, which is largely dominated by *Larrea tridentata* and *Ambrosia dumosa* covers up to 70% of the landscape (Rowlands et al. 1982, Germano et al. 1994, USFWS 1994). This association occurs below 1,500 m on alluvial fans and bajadas. On the upper slopes, a succulent scrub association dominated by stem succulent species, including *Cactaceae* and *Yucca*, can be common (USFWS 1994). Different combinations of plant associations occur in each desert region and some unique plant communities occur in localized areas. For example, the Mojave saltbush-allscale scrub community (dominated by *Atriplex spinifera* and *A. polycarpa*) only occurs in the Western Mojave Desert near Fremont Peak and Kramer Junction, CA (Rowlands et al. 1982, USFWS 1994). The Northern Mojave Desert is a transitional vegetation zone with a combination of plants common to the Mojave Desert and the Great Basin Desert (Rowlands et al. 1982). The Colorado Desert contains a unique combination of Sonoran Desert and Mojave Desert flora (Burk 1997).

Desert tortoises occur in a variety of habitat throughout their range. Desert tortoises have been observed from below sea level to 2,225 m, though most tortoise observations are documented between 300 m and 900 m (Luckenbach 1982). In the Mojave and Colorado Deserts, desert tortoises most commonly occur in areas with gentle slopes, sufficient shrub cover, and friable soils to allow burrow construction (Bury et al.

1994, Andersen et al. 2000, USFWS 2008). Although desert tortoise habitat could be considered fairly contiguous in the Mojave and Colorado Deserts, the existence of large mountain ranges, such as the New York and Providence Mountains in California and the Spring Mountains in Nevada, low elevation playas, and a variety of other physical features, are potentially formidable barriers to gene flow. Habitat fragmentation caused by humans, including urban development and interstate freeways, may also impact movement dynamics.

## **METHODS**

### **Sampling Design**

Our study was designed to sample potential intra- and inter-population genetic variability. However, boundaries to potential desert tortoise populations are not apparent, and sampling design is critically important as a means to identify population boundaries successfully. To study genetic diversity, gene flow, and population genetic structure, it is important to collect samples from the entire geographic and ecological range of the species (Lowe et al. 2004). For analyses, we assumed that there is no gene flow between the Mojave population and Sonoran population, which is located east and south of the Colorado River. These two populations apparently have been separated for 5.5 million years based upon analyses of mitochondrial DNA (Lamb et al. 1986, Lamb and McLuckie 2002, Edwards et al. 2004).

Whole blood was collected from 748 desert tortoises throughout the Mojave and Colorado Deserts between 2004 and 2006 (Table 1, Fig. 1). These samples were grouped

subjectively into 25 sampling locations that were considered to be a specific geographic area, often constituting one or two valleys, and reflecting geography and political boundaries (Table 1 provides a brief description of locations).

Sampling design differed based on land ownership and density of tortoises; however, efforts were made to sample evenly from all potential populations and to collect at least 30 samples from each geographic location where possible. We chose our sample sizes based on previous simulation studies in the literature. Under most conditions, sampling 20 - 25 individuals with the use of more than ten microsatellite markers provided sufficient power to detect population structure (Evanno et al. 2005, Waples and Gaggiotti 2006). Approximately half of DNA samples ( $N = 350$ , 46.8 %) used in this study were collected along randomly-placed transects during routine population monitoring conducted by the U.S. Fish and Wildlife Service (USFWS 2006). A small percentage of individuals ( $N = 11$ , 1.5%) were sampled opportunistically while technicians were en route to a transect. The remaining samples ( $N = 387$ , 51.7%) were collected from efforts not associated with population monitoring between 2004 and 2006. Some of these samples were collected along random transects within the Piute and Eldorado Valleys and from animals tracked with radio transmitters in those valleys, and other samples were collected from transects (4-12 km) placed systematically to cover poorly sampled areas of the range. Many of these sampling transects were located outside of desert tortoise critical habitat, which are the areas that are actively managed for recovery by the U.S. Fish and Wildlife Service (Fig. 1). We sampled in both types of areas to cover as much of the range as possible and to determine more effectively the

locations of genetic boundaries for populations. Transect sampling was employed to minimize the probability of sampling within closely related groups of tortoises (demes).

#### Sample Collection, DNA Isolation, and Genotyping

Whole blood from 748 desert tortoises was dried onto dots of filter paper, and stored until DNA could be isolated from the samples. Total genomic DNA was extracted from up to three filter-paper dots using a dried blood protocol for QIAGEN DNeasy kits (Qiagen 2001). DNA was eluted in a TE buffer, quantified using a Labsystems Fluoroskan Ascent fluorometer, and diluted to concentrations between 5-10 ng/ $\mu$ l for amplification with microsatellite loci.

DNA was amplified using the polymerase chain reaction (PCR) and genotyped with 20 microsatellite markers. Six microsatellite primer sets (GP15, GP30, GP61, GOAG3, GOAG4, GOAG7) were obtained from previous studies of *Gopherus polyphemus* (Schwartz et al. 2003) and the Sonoran population of *Gopherus agassizii* (Edwards et al. 2003). An enriched microsatellite library, developed by Genetic Identification Services, was used to identify 14 additional microsatellite primers sets (Hagerty et al. 2008, Appendix A). All microsatellite loci were amplified in six multiplex PCRs and two individual PCRs. All multiplex reactions contained ratios of primer concentrations that were determined by trial and error. Multiplex PCRs contained 1x Multiplex PCR Master Mix (QIAGEN), 0.2  $\mu$ l multiplex primer cocktail, and 60 - 80  $\mu$ l genomic DNA in a 16  $\mu$ l PCR reaction. Multiplex 1 ( $T_a = 57^\circ\text{C}$ ) contained primers GOAG7 and GOAG3. All multiplex PCR cycling was performed using a MBS Satellite

0.2G thermal cycler with the following profile: 1 cycle of 94°C for 15 min, 33 cycles of 94°C for 30s, appropriate annealing temperature for 90s, 72°C for 30s, and 1 cycle of 62°C for 30min. Multiplex 2 ( $T_a = 55^\circ\text{C}$ ) contained primers GP61, GP30, and GP15. PCR conditions were identical to Multiplex 1. The remaining multiplex reactions for GOA1, GOA2, GOA3, GOA4, GOA6, GOA8, GOA9, GOA11, GOA12, GOA13, GOA14, GOA22, and GOA23 were completed as described in Hagerty et al. (2008).

GOAG4 and GOA17 were amplified as single PCRs. The 15- $\mu\text{l}$  reactions contained 1x Titanium taq PCR buffer (pH 8.0, 3.5mM  $\text{MgCl}_2$ ) (CLONTECH Laboratories, Inc.), 0.2 units Titanium taq DNA polymerase (CLONTECH Laboratories, Inc.), 0.25 mM dNTPs, 0.2  $\mu\text{M}$  forward and reverse primer, and 60-80  $\mu\text{l}$  genomic DNA. Cycling conditions were 1 cycle of 94°C for 1min, 33 cycles of 94°C for 30s, 61°C (GOA17) or 55°C (GOAG4) for 30s (annealing), 72°C for 30s, and 1 cycle of 72°C for 30min.

All amplified microsatellite segments underwent a multi-color fluorescence-based DNA fragment size analysis in five separate panels using a fully automated ABI 3730 DNA sequencer. We amplified microsatellites and completed fragment analysis in collaboration with the Nevada Genomics Center (<http://www.ag.unr.edu/Genomics/>). All alleles were scored with GeneMapper 5.0 (Applied Biosystems).

### Descriptive Population Genetic Analyses

Descriptive statistics, including observed heterozygosity and expected heterozygosity and number of alleles per locus, were calculated using GENETOP

(Raymond and Rousset 1995). Tests for linkage disequilibrium for each pair of loci, and deviation from Hardy-Weinberg equilibrium, were performed in FSTAT (version 2.9.3.2, February 2002; Goudet 2001). All loci that were significantly linked to another locus consistently across all sampling locations were removed from subsequent analyses. We also performed a test for null alleles in MICROCHECKER (version 2.2.3; van Oosterhout et al. 2004). If the combined probability of expected heterozygote classes ( $P < 0.05$ ) was significant consistently across sampling locations, we removed the locus from analyses. An estimate of  $F_{IS}$  was calculated for each locus and across loci for each sampling location to test for significant heterozygote deficits, which would indicate a deviation from Hardy-Weinberg equilibrium. We tested for statistical significance using  $\alpha = 0.05$ , and we controlled for multiple testing using the Bonferroni correction (Rice 1989).

### Identifying Subpopulations

We investigated the genetic population structure of the desert tortoise in the Mojave and Colorado Deserts using two Bayesian clustering models. Program STRUCTURE (version 2.1; Pritchard et al. 2000, Falush et al. 2003, Pritchard et al. 2007) was used to infer the number of genotype clusters without a priori knowledge about potential population clusters. Program GENELAND (Guillot et al. 2005b) has similar assumptions and uses a similar resampling algorithm, but also incorporates spatial data for each individual into the analysis.

### STRUCTURE Procedures and Parameters:

STRUCTURE uses a Bayesian statistical approach to define the number of distinct breeding groups ( $K$ ) based upon the probability of multilocus genotypes given the allele frequency data. The most likely number of genotype clusters is determined as the number of distinct groups in linkage equilibrium and Hardy-Weinberg equilibrium (i.e., characterizing a randomly mating population). The variables of the model (genotype cluster of origin, and allele frequencies of each cluster) are estimated using a Markov Chain Monte Carlo (MCMC) re-sampling algorithm over a range of possible clusters ( $K$ ) (Appendix 2). We used an admixture model, which allows for multiple origins of individuals, with correlated gene frequencies (Falush et al. 2003), and we used a uniform prior, making assignment to each  $K$  equally likely. We specified a 750,000 MCMC burn-in period followed by ten 750,000 MCMC replicates per  $K$ , from  $K = 1$  to  $K = 10$ , to approximate the posterior allelic distributions against which individual genotypes were compared and assigned to a cluster (Pritchard et al. 2000). We ran initial simulations which suggested that  $K > 10$  were unlikely.

For each value of  $K$ , an estimate of the posterior probability of the model fit,  $Pr(X / K)$ , was used and the best fit was determined from the “estimated natural log of the probability of data” or  $Ln P(D)$  (Appendix 2). We calculated the mean  $Ln P(D)$  and standard deviation around the estimate from the 10 iterations per  $K$ . Inferring the most probable number of genotype clusters,  $Pr(X / K)$ , is not straightforward, and can only be approximated using ad hoc procedures (Pritchard et al. 2000, Pritchard et al. 2007). The most probable number of clusters is taken to be the value at which the estimate of  $ln Pr(X / K)$  (or  $ln P(D)$ ) is highest, and the value of  $K$  that maximizes consistency among the

parameter of individual admixture ( $\alpha$ ) (Pritchard et al. 2007). Thus, the smallest value of  $K$  explaining the structure in the data well is taken to be the most likely solution. The second-order rate of change in the posterior probability ( $\Delta K$ ) has been advocated as a more reliable statistic to determine the appropriate  $K$  (Evanno et al. 2005). This more formal criterion uses the largest change in the slope of the distribution of  $\ln P(D)$  as an indication of the most likely  $K$  (Appendix 2). We compared the results of each of these ad hoc criteria, in conjunction with other basic diagnostics such as the value of the admixture parameter ( $\alpha$ ) and the pattern of assignment to clusters, to estimate the true number of genotype clusters (Evanno et al. 2005, Pritchard et al. 2007). In all cases, additional information about the biology of the species, and consistency among runs, is necessary to make inferences.

Simulations of populations with more complicated structural organization than an island model indicate that the  $\Delta K$  statistic often identifies the uppermost level of structuring among potential populations (Evanno et al. 2005). We used additional STRUCTURE simulations with similar parameter values to detect any potential sub-structuring within the clusters identified by the initial model simulations. We used the individual assignments from the number of clusters identified using  $\Delta K$  to create data sets ( $N = K$ ), and we searched for additional hierarchical structuring within each basal cluster using STRUCTURE (Evanno et al. 2005, Rowe and Beebe 2007, Pritchard et al. 2007). We continued to analyze subsequent clusters until the model did not support additional subdivision.

Unequal sample sizes across the entire distribution of sample effort may lead to the inference of more clusters than those actually occurring. Spuriously inferred clusters

also could result due to the inability of STRUCTURE to delineate clusters in an effectively well-mixed region (McRae et al. 2005, Pritchard et al. 2007). To account for potential bias in the number of populations inferred from STRUCTURE caused by unequal sampling among locations, we reduced the number of genotypes in locations that had more than 30 sampled individuals. We randomly selected 30 individuals from each location and used the reduced number of genotypes to infer the number of genotype clusters. Locations with less than 30 individuals remained unchanged in the analyses. Individual genotypes were re-sampled with replacement to produce ten replications of the analysis. Procedures as described above were completed for each replicate and compared for consistency. Hierarchical analyses were also completed with reduced data sets.

#### GENELAND Procedures and Parameters:

Program GENELAND implements a Bayesian clustering algorithm similar to STRUCTURE, and also uses an MCMC re-sampling method to estimate unknown parameters including the number of genotype clusters. However, GENELAND additionally incorporates spatial data (geo-referenced coordinates) for each individual (Guillot et al. 2005b). GENELAND uses an hierarchical strategy, inferring genetic structure of populations based upon the spatial organization of the populations. Thus, an additional assumption in this model is that populations are spatially organized as a set of non-overlapping polygons with no gaps through the colored Poisson-Voronoi tessellation (Guillot et al. 2005a, 2005b). One key difference between STRUCTURE and GENELAND is that the number of clusters must be inferred using ad hoc approximations

in the former, but the number of genotype clusters is treated as a parameter and processed in GENELAND (Guillot et al. 2005a).

Four individuals were removed from GENELAND analyses because we did not have reliable spatial coordinates for them. GENELAND simulations were performed with the GENELAND GUI in the R-PACKAGE. In our simulations, we used spatial (spatial = TRUE) and genetic data (Dirichelet model of allele frequencies) as a priori information. We included uncertainty (1 km) into the spatial coordinates for each individual to account for any measurement error, movement of individuals, and the potential for observed locations to reflect the true locations inaccurately (Guillot et al 2005a, Coulon et al. 2006). The first set of MCMC chains was used to determine the modal number of inferred populations (as suggested in Guillot et al. 2005a). The MCMC algorithm was repeated 10 times (allowing  $K$  to vary among simulations) using the following parameters: (1) minimum number of populations was 1, (2) initial number of populations was 2, (3) maximum number of populations was 15, (4) 500,000 MCMC iterations, (5) 10 thinning (saving only 1 iteration per 10), (6) maximum number of nuclei in the Poisson-Voronoi tessellation was 300 (default), and (7) maximum rate of Poisson process was 100 (default). After the modal number of populations ( $K$ ) was estimated from the initial 10 simulations, the previously inferred value of  $K$  was used as the initial and maximum number of populations in five additional runs with the same model parameters. Mean assignment probabilities were calculated for each individual from the five runs. During post-processing, we used 200 pixels along the X-axis and Y-axis and a burn-in of 1,000 MCMC cycles. The model identified the modal population of each individual and the probability of assignment of each individual to the modal population. Hierarchical

clustering was evaluated using this model with the same method described for STRUCTURE.

#### Statistics for Inferred Subpopulations:

First, we determined population differentiation among all sampling locations. Then, we estimated population differentiation among the populations from the two Bayesian techniques. If the two techniques provided different population boundaries, those boundaries were compared. Pair-wise  $F_{ST}$  values (Weir and Cockerham 1984) were calculated for all combinations of inferred populations and sampling locations in program FSTAT 2.9 (Goudet 2001). We tested for pair-wise genetic differences among clusters and sampling locations (not assuming Hardy-Weinberg equilibrium) using a permutation test that randomized genotypes across populations and created new data sets that are evaluated with the log-likelihood statistic  $G$  (Goudet et al. 1996). Statistical significance ( $\alpha = 0.05$ ) was evaluated after the Bonferonni correction for multiple comparisons (Rice 1989). An analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was also conducted to test the significance of the inferred population structure, which was implemented in Arlequin 2.0 (Schneider et al. 2000). Finally, a frequency-based assignment approach, implemented in DOH, was used to evaluate the hypothesis of genotype clusters provided by the Bayesian approaches (Paetkau et al. 1995).

Mantel tests were used to test for correlation between genetic distance and geographic distance matrices using the web-based package, Isolation by Distance, web service (IBDWS, Jensen et al. 2005). We tested for isolation by distance among sampling

locations using pair-wise geographic distances and  $F_{ST} / (1 - F_{ST})$  (Rousset 1997). The geographic distance matrix was developed for all sampling locations using ArcGIS 9.0 (ESRI). Straight-line distances between the centroids of sampling locations were used as an estimate of geographic distance between locations.

## RESULTS

### Descriptive Population Genetic Analyses

Average heterozygosity (gene diversity) of the Mojave desert tortoise ranged from 0.643 in Gold Butte to 0.799 in Southeast Las Vegas Valley ( $\bar{X} = 0.742 \pm 0.040$ ) (Table 2). These values of gene diversity and allelic richness ( $\bar{X} = 8.352 \pm 3.354$ ) provided high levels of diversity to investigate genetic structure within the sampling area.

Although some pairs of loci were statistically linked (i.e.  $P < 0.05$ ), these pairs were not statistically linked in multiple sampling locations. If a pair of loci was in a state of linkage disequilibrium, that pair should be linked in several populations. The pairs of microsatellites were not linked statistically in multiple locations, reducing the chance that the loci violated the assumption of linkage equilibrium. Pairs of microsatellites did not exhibit significant linkage disequilibrium among locations or in any particular group after the Bonferroni correction ( $P < 0.000011$  after 95,000 permutations). Therefore, we did not remove any microsatellites due to a violation of the assumption of independence.

Six of the 25 sampling locations (GB, MD, EL, PI, CM, EP; see Table 1 for abbreviations) had significant  $F_{IS}$  values after the Bonferroni correction ( $P < 0.0001$ ) (Table 2), indicating that these sampling locations are not in Hardy-Weinberg

equilibrium. However, these significant values were influenced by two loci (GOA 6 and GP 61; Appendix B). If these two loci were influencing the  $F_{IS}$  values due to problems with amplification, they would cause high  $F_{IS}$  values in several, if not all of the sampling locations. It was likely that each sampling location did not represent a discrete randomly mating population, which would create conditions outside of Hardy-Weinberg Equilibrium. The test for the presence of null alleles complemented the  $F_{IS}$  values. GOA6, GOA9, GOA12, and GP61 had multiple populations (between 6 - 9 locations) with a significant combined probability of expected heterozygote classes ( $P < 0.05$ ). However, this evidence for the presence of null alleles did not occur consistently across all tested locations, and we chose to retain those loci for the subsequent analyses.

### Identifying Subpopulations

#### Bayesian Clustering without Spatial Information (STRUCTURE):

Using Program STRUCTURE with a sample size of 748 tortoises,  $\ln P(D)$  across 10 independent runs reached a plateau after  $K = 9$  (Table 3). This inflection point indicated that nine clusters are more appropriate than ten clusters despite a slight increase in the posterior probability. The proportion of admixture ( $\alpha$ ) also was lowest and reached a plateau at  $K = 9$  (Table 3). However, the largest increase in the likelihood that a model was a good fit occurred between  $K = 1$  and  $K = 2$ .  $\Delta K$ , which measures the second order rate of change between  $K$  and  $K-1$ , also peaked when  $K = 2$  (Table 3; Fig. 2a). Several independent runs of STRUCTURE for  $K = 1$  never converged, thus, it may be inappropriate to compare STRUCTURE results for  $K = 1$  with results using other values

of  $K$ . When we removed  $K = 1$  from analysis to find the best fit to the data,  $K = 3$  became the most probable configuration because the probability of fit became much higher between  $K = 2$  and  $K = 3$ . The  $\Delta K$  for  $K = 3$  was at least two times higher than  $\Delta K$  for subsequent values of  $K$  (Table 3; Fig. 2a). A large reduction in the admixture parameter ( $\alpha$ ) also occurred between  $K = 2$  and  $K = 3$  (Table 3).

Multimodality in the model fit prohibited clear interpretation of our data set. At least two local maxima were reached within different independent MCMC simulations for each  $K$  value (not shown). Different local maxima for independent simulations occurred when  $K \geq 4$ . Multimodality did not occur when  $K$  was less than 4 or when  $K = 9$ , which was also the model with the highest mean  $\ln P(D)$ .

We chose  $K = 3$  as the basal, most parsimonious number of genotype clusters because of the high  $\Delta K$  and because of the occurrence of multimodality for runs when  $K$  was greater than three (Fig. 2a). This level of clustering was interpreted to represent the uppermost level of clustering across the landscape. Proportional membership for each sampling location to one of the three clusters was high and ranged from 62 to 97%. Cluster 1 (Northern Mojave or NM) encompassed seven sampling locations in Utah and Nevada (RC, BD, MM, GB, MD, CS, NEL; Fig. 2b). The transition between cluster 1 and cluster 2 occurred gradually across several mountain ranges such as the Arrow Canyon Range, which extend North to South and are potential partial barriers among locations north and east of Mormon Mesa to areas south and west of Mormon Mesa. Cluster 2 (Las Vegas or LV) encompassed 9 sampling locations in Nevada and along the Nevada/California border (NWL, AM, PA, SH, IV, SI, SWL, SEL, EL; Fig. 2b). A transition zone between cluster 2 and cluster 3 was apparent across Searchlight Pass, a

connection point for the Eldorado, Newberry, and Highland ranges. This low pass (1500 m) separates Eldorado and Piute Valleys near the Nevada/California border. Cluster 3 (California or CA) contained individuals from the remaining nine sampling locations in California and in Piute Valley, Nevada (PI, CM, EP, WP, CK, PM, OR, SC, FK; Fig. 2b).

We examined the potential for hierarchical sub-structuring within each of the three basal clusters. Hierarchical sub-structuring could explain the ostensible discrepancy between  $\Delta K$  and the peak mean  $\ln P(D)$ . Each cluster had an additional level of structuring. Cluster 1 (NM) was divided further into two clusters (Table 4). Cluster 2 (LV) was divided further into three clusters (Table 4). Cluster 3 (CA) was divided further into four clusters (Table 4). The additional clusters that were identified in the hierarchical analyses aligned exactly with the clusters identified by the model when  $K = 9$  (Fig. 3), providing evidence that some additional level of structure does exist within the Mojave desert tortoise. Proportional membership of sampling locations to each of the nine clusters from the complete analysis was variable (Fig. 3). Although, several locations were clearly assigned to a particular cluster (e.g. proportional membership of Mormon Mesa to cluster 1 was 88%), others were split among clusters (e.g. proportional membership of Ivanpah Valley to each cluster was  $< 30\%$ ; Fig 3). When  $K = 2$  was chosen as the most basal number of clusters and used to investigate sub-structuring, the resulting clusters were identical (not shown). Additional sub-structuring was not present in any of the nine genotype clusters when they were analyzed separately (not shown).

Finally, we investigated the potential for unequal sampling effort to influence model choice for the number of genotype clusters. When sampling effort was more evenly distributed among locations (with  $N \leq 30$ ),  $\ln P(D)$  peaked when  $K = 6$ . The reduction in

the number of genotype clusters when  $K = 6$  resulted in no subdivision of the “California” cluster (Fig. 4). Using all of our data, the “California” cluster was split into additional clusters.  $\Delta K$  with the reduced dataset was also  $K = 3$ , and these three basal clusters were identical to those identified with the full data set. With the reduced data set, the resulting number of individuals assigned to each of these three basal clusters was similar ( $N_1 = 165$ ,  $N_2 = 212$ ,  $N_3 = 208$ ). The reduction in sample size also lowered the total number of genotype clusters identified in hierarchical clustering analyses to seven. However, these differences did not align with results of the full model using the reduced dataset where  $\ln P(D)$  was highest at  $K = 6$ , as they did in the previous analyses (i.e. clusters identified using a hierarchical analysis with the complete data set were identical clustering when  $K = 9$ , but hierarchical results were not the same as best fit of the full model with the reduced dataset). The sub-structuring for cluster 1 (NM) remained unchanged (two clusters). However, cluster 2 (LV) was only subdivided into two clusters, which removed the Eldorado Valley cluster (Fig 5a). Cluster 3 (CA) was subdivided into three clusters, which removed the Piute Valley cluster (Fig 5b).

#### Bayesian Clustering with Spatial Information (GENELAND):

Spatial information was included as a priori information to infer population boundaries using Program GENELAND.  $K = 4$  consistently resulted as the modal number of genotype clusters, though  $K = 3$  was chosen twice out of 10 simulations (Table 5). Two of the four genotype clusters were similar to those resulting from analyses using STRUCTURE when  $K = 3$ . The Northern Mojave cluster and the Las Vegas Cluster were delineated with similar boundaries to those identified without the spatial information in

the STRUCTURE analyses (Fig. 6). The California cluster, identified by STRUCTURE, was split into two clusters in the GENELAND analyses (Fig. 6). The West Mojave was separated from the remainder of the California sampling locations (Eastern Colorado sites, Northern Colorado sites) (Fig. 6). Assignment to these clusters was consistently greater than 90%. When the model was constrained to  $K = 3$ , a majority of independent simulations (4 out of 5) identified the same three clusters identified by STRUCTURE. Hierarchical structuring was not detected in the NM or LV cluster; however, the Eastern and Northern Colorado separated as hierarchical clusters within the CA cluster in subsequent analyses.

#### Statistics for the Inferred Subpopulations and Sampling Locations

The Bayesian clustering methods did not provide consistent, definitive delineations for population structure. Therefore, we tested multiple configurations of genotype clusters using an analysis of molecular variance (AMOVA). We compared  $K = 3$ ,  $K = 7$ , and  $K = 9$  from STRUCTURE and  $K = 4$  from GENELAND. In all cases, the amount of variation explained by differences among population was low ( $< 5\%$ ), and most genetic variation was explained by differences within populations (more than 80%) for all configurations ( $K = 3$ ,  $K = 4$ ,  $K = 7$ ,  $K = 9$ ; Table 6). However, all variance components, including the among-population portion, contributed significantly to the genetic variation among clusters ( $P < 0.0001$ ).

Pair-wise  $F_{ST}$  values (Weir and Cockerham 1984) among sampling locations ranged from 0.003 (Chemehuevi - East Providence Mountains) to 0.162 (Beaver Dam

Slope– Pinto Mountains) (Appendix 2). Almost all pair-wise comparisons for population differentiation were significant after Bonferroni correction ( $P < 0.000167$  after 6000 permutations), except for a few locations that were in close proximity (adjacent locations). Southeast Las Vegas had a small sample size ( $N = 12$ ), which likely affected significance values for several pair-wise comparisons that were not directly adjacent (Appendix 2). Similar to the AMOVA results,  $F_{ST}$  values suggest that only a very small amount of genetic variation results from population substructure, except for the most distantly paired locations, which had greater yet still only moderate levels of differentiation. When locations were combined to correspond to the 7 or 9 inferred genotype clusters from STRUCTURE, pair-wise  $F_{ST}$  values ranged from 0.012 (Amargosa – South Las Vegas) to 0.132 (Virgin River – Eastern Colorado), and they followed a pattern similar to comparisons among all sampling locations (Table 7). Each pair-wise comparison for genetic differentiation was statistically significant after Bonferroni correction ( $P < 0.001389$  after 720 permutations).

Self-assignment of individuals to sampling locations was variable (7.14% - 89.1%). However, the percentage of self-assignment improved dramatically when sampling locations were clustered to resemble the inferred populations ( $K = 9$ ; 64.7% - 92.4%; Table 8). Additionally, no random assignments occurred in any of the 7 or 9 populations after 10,000 re-sampling events.

Isolation by distance was evident across the range of the Mojave desert tortoise (Fig. 7). Genetic and geographic distances among sampling locations were correlated strongly ( $Z = 4392.398$ ,  $r = 0.824$ ,  $P < 0.0001$ ).

## DISCUSSION

### Identifying Meaningful Genotype Clusters for the Mojave Desert Tortoise

Conservation and management actions for the Mojave desert tortoise are implemented in a spatial context, using previously delineated conservation units (called Recovery Units; USFWS 1994). Multiple forms of evidence, including previously completed population genetic studies, were used to establish these delineations. Our main goal in the research reported here was manifold. First, we used recent advances in molecular techniques, and analysis of population genetic data, to determine to what extent the Mojave desert tortoise exhibits population structure. Second, we evaluated how closely do potential genetic populations reflect current understanding of the population biology. Finally, we wanted to use new insights into the genetics of desert tortoise to translate biology into justifiable management practices.

The interface among natural populations typically is complicated and involves variable rates of gene flow among demes that display structure at multiple scales (Hanski and Gilpin 1997, Hey and Machado 2003, Manel et al. 2003). Many species do not display clear population structure resulting from present geographic or ecological barriers, or as a result of historical interactions (e.g., Sponer and Roy 2002, Spinks and Shaffer 2005, Pilot et al. 2006). Often these species have large distributions, and they have the ability to disperse over large geographic regions (e.g., caribou, Boulet et al. 2007; cougars, McRae et al. 2005; grey wolves, Pilot et al. 2006; lynx, Rueness et al. 2003). This situation can be contrasted to species with an obvious potential for stark genetic differentiation such as amphibians whose close physiological relationship with

water can limit dispersal, especially in desert regions where amphibian habitat is separated by formidably desiccating desert environments (Bradford et al. 2003, Simandle 2006). Recent developments in the analysis of genetic data using assignment tests do not require an a priori definition of what constitutes a population (Manel et al. 2003, Beaumont and Rannala 2004, Manel et al. 2005). These analyses offer the potential for improved understanding of population structure for species lacking definitive boundaries (Pritchard et al. 2000, Falush et al. 2003, Guillot et al. 2005b). This approach is ideal to investigate population structure in the Mojave desert tortoise.

Using two Bayesian clustering methods implemented in STRUCTURE (Pritchard et al. 2000) and GENELAND (Guillot et al. 2005b), we successfully identified spatial structure in the Mojave desert tortoise. The gamut of STRUCTURE results consistently identified three basal desert tortoise populations in the Mojave assemblage, using the  $\Delta K$  metric (Evanno et al. 2005). Previous analyses of simulated data have demonstrated that STRUCTURE is able to identify the uppermost level of structuring for migration models that are more complex than the traditional island model, which is presumably common for most species (Evanno et al. 2005). Identical cluster boundaries were identified in a majority of the trials when the spatial Bayesian model (GENELAND) was constrained to  $K = 3$ . However, the addition of spatial context also highlighted the Western Mojave as a separate cluster in the modal number of genotype clusters (four clusters). The differences between these two clustering methods, and how this affects interpretation of inferred genotype clusters, will be discussed below.

Across the range of the Mojave desert tortoise, the three basal clusters follow a north to south gradient (Fig. 1). The Northern Mojave cluster is comprised of sampling

locations in the northern part of the range (as far north as St. George, UT) with localized transitional zones between Mormon Mesa and Coyote Springs (through Moapa Valley) and across the Muddy and Virgin Rivers. This area is topographically complex and most likely provides a mosaic of available habitat for tortoises, interspersed among mountain peaks taller than 1,000 m.

Prior to extensive urban development, Las Vegas Valley provided a continuous tract of tortoise habitat with open corridors to the northwest and south. Previously, researchers hypothesized that this area was a transitional corridor between locations to the north and south (Britten et al. 1997). As a result of this historic potential for connectivity, the Las Vegas Cluster is extensive, including locations as far northwest as Oasis Valley in Nevada and southeast to Eldorado Valley, NV. The transitional area between the Las Vegas cluster and the California cluster occurs in two main areas: across Searchlight Pass between the Eldorado and Piute Valleys and across the major montane barriers of the New York and Providence Mountains, which bisects the Mojave National Preserve. Additionally, these montane barriers were clearly identified with GENELAND. The California cluster contains most of the Mojave and Colorado Deserts in California, except for transitional zones occurring near the California and Nevada border. The habitat in this cluster is relatively continuous, despite the occurrence of several different vegetation communities.

The three basal genotype clusters inferred with microsatellite markers (Northern Mojave, Las Vegas, and California) closely resemble the distribution of the three mtDNA haplotypes found previously (Lamb et al. 1989). These haplotypes had very few restriction length differences in comparison to the Sonoran and Sinaloan haplotypes;

less than 0.5% nucleotide differences were found among Mojave haplotypes (Lamb et al. 1989). Recent mtDNA sequencing also corroborated this low divergence rate among Mojave haplotypes (Edwards 2003, Murphy et al. 2007). Two major mtDNA lineages were identified (Murphy et al. 2007) and these lineages correspond to genotype clustering using microsatellite data in this study. The combination of microsatellite and mtDNA evidence provides support for the existence of population structure at the landscape scale, despite a weak mtDNA signal. Inferences from our study were made without any presuming underlying population structure, and they complement mtDNA results from previous research. The small amount of mtDNA divergence within the Mojave desert tortoise suggests recent divergence within this group, especially when contrasted to divergence between the Mojave and Sonoran desert tortoises. These groups likely diverged approximately 5 million years ago (Lamb et al. 1989, Lamb and McLuckie 2002, Edwards et al. 2004) and may constitute separate species (Berry et al. 2002, Murphy et al. 2007).

Previous studies using simulated and real data sets successfully identified fine scale structure in complex systems using hierarchical clustering methods (Evanno et al. 2005, Rowe and Beebee 2007). When we separated and reanalyzed the basal clusters (2 or 3) to detect any hierarchical sub-structuring, we identified structure at a finer geographic scale within each cluster. Fine-scale delineations highlighted by hierarchical analyses were also apparent in the number of genetic populations when the complete data set was analyzed. A majority of these delineations were robust to the random removal of individuals used to create an equal sampling effort across the range. However, locations with intense sampling (e.g., Piute Valley) did appear to increase the chance that the

models identified additional, potentially spurious clusters. When the sample size was experimentally reduced to assess the importance of sampling design, locations that were deemed to be distinct using the full data set no longer separated as distinct genotype clusters (this was particularly the case in the hierarchical analyses). For example, both Eldorado Valley and Piute Valley were not identified as distinct clusters when the sample size was reduced for those locations. Therefore, we are skeptical that some of the finer delineations of clusters are anything more than artifact of sampling design, and not likely biologically meaningful. Our results highlighted the reported effect of sampling intensity on the STRUCTURE model that was found in a previous study (McRae et al. 2005). We emphasize the importance of sampling design in studies intended to identify population boundaries, as well as careful interpretation of results from Bayesian clustering methods.

Fine scale clustering using these data are relevant to previous hypotheses of the structure of desert tortoise populations in the listed portion of the range. Prior to the original Recovery Plan for the desert tortoise (USFWS 1994), few genetic data were available to distinguish among regions in Nevada and Utah. Despite morphological, ecological, and behavioral differences among tortoises in the current Upper Virgin River recovery unit located entirely in north of the Beaver Dam mountains (USFWS 1994), we found no genetic evidence that this area is distinct from adjacent location along the Beaver Dam Slope (the southern face of the Beaver Dam Mountains), further south in Utah and into Nevada (Mormon Mesa). Previous mtDNA, allozyme, and morphometric data from the original Northeastern Mojave recovery unit (Britten et al. 1997) lead to the hypothesis that additional variation existed in this region and that original conservation units did not reflect this diversity.

Indeed, we detected genotype clusters within this region, supporting the Britten et al. hypothesis. Four genotype clusters were apparent in the current Northeastern Mojave recovery unit. The Virgin River cluster split and transitioned into a Muddy Mountains cluster. Additionally, the northern portion of Las Vegas Valley (including Coyote Springs Valley) was separated from the southern portion of Las Vegas Valley (including Eldorado Valley). The Amargosa Desert, Oasis Valley, Pahrump Valley, Greenwater Valley (in Death Valley National Park) and Shadow Valley also formed the distinct Amargosa cluster. These locations were outliers in a previous analysis (Britten et al. 1997); however, sampling locations in California (some of which clustered with this group) were not included in the Britten et al. study. We were able to detect cluster boundaries by sampling randomly and extensively across the range of the Mojave assemblage.

Habitat differences driven by variation in climate (predominantly rainfall) as well as correlated behavioral and life history differences were used previously to distinguish among regions within the California cluster (Peterson 1994, USFWS 1994, Peterson 1996, Henen et al. 1998, Lovich et al. 1999, Wallis et al. 1999, Tracy et al. 2004). We identified three groups within the basal California cluster. The Northern Colorado cluster, which also contains Piute Valley, borders the South Las Vegas Cluster and transitions to that cluster at the Searchlight Pass. The Eastern Colorado cluster is the most southern cluster in the assemblage, and the Baker Sink separates it from the Northern Colorado cluster. The Baker Sink is part of a potential low-elevation barrier extending from Saline Valley in California in the north, then south through Death Valley, Silurian Valley, Baker Sink, and Cadiz Valley. This barrier reflects the formidable effects of the lower

elevations and extremely hot climates along this line, which divides the ecological western Mojave Desert, with its quite variable winter-spring precipitation regime, lower elevations, and Mojave River hydrology, from the more eastern Mojave Desert, subject to more predictable winter and summer monsoon precipitation, more variable elevations, and closed basin and Colorado River hydrology (Tracy et al. 2004). The Western Mojave cluster is separated from the Eastern Colorado cluster in the Pinto Mountains, and from the Amargosa cluster in the low elevation area near Death Valley. The Western Mojave cluster was also highlighted as a distinct cluster using spatial data in GENELAND. We found no additional hierarchical clustering within the Western Mojave cluster, which is not consistent with another recent study using microsatellites to delineate desert tortoise subpopulations (Murphy et al. 2007). We address inconsistencies between these two studies below. In summary, we identified three basal genotype clusters that were further delineated into seven groups at a finer scale (Fig. 1).

#### Limitations to Identifying Desert Tortoise Subpopulations

Despite the inferred presence of broad and fine scale population structure identified by the Bayesian analyses, only low genetic differentiation was detected among most sites using F-statistics. Moderate differentiation occurred only among the most geographically distant sites, and great differentiation occurred nowhere.  $F_{ST}$  values provide a summary statistic that describes the result of cumulative gene flow across multiple generations, and these statistics do not allow us to differentiate among different hypotheses for population dynamics (i.e. reflecting historically moderate to high levels of

gene flow that no longer occur, or reflecting current gene flow; Neigel 2002, Pearse and Crandall 2004).

To explore past demography, coalescent-based methods may provide useful estimates of population parameters (Beerli 1998, Beerli and Felsenstein 1999, 2001, Nordborg 2001, Pearse and Crandall 2004). Estimates of long-term gene flow can be complemented by assignment methods, which can detect recent gene flow and potentially first generation migrants (Paetkau et al. 2004, Manel et al. 2005). The current level of habitat fragmentation, and the isolation of critical habitat, supports an hypothesis of historically high levels of gene flow. In addition, the majority of genetic variation can be explained by differences among individuals within populations (as determined by the AMOVA). Although the amount of genetic variation explained by population structure is significant, the percentage of variation explained was small relative to individual variation.

The low to moderate levels of genetic differentiation seen among desert tortoise populations also follows a gradient that could be consistent with isolation-by-distance (Wright 1943). Isolation-by-distance is one of the simplest models explaining differentiation among populations in the absence of barriers to gene flow (Wright 1943, Kimura and Weiss 1964, Slatkin 1993). Typically, genetic distance increases with geographic distance where the dispersal ability of the species limits interactions among individuals beyond the local scale in comparison to the whole range (Kimura and Weiss 1964, Slatkin 1993, Manel et al. 2003). The Mojave desert tortoise exhibits a strong correlation between geographic distance and genetic distance; geographic distance explained 65% of the variation in genetic distance among sampled locations. These

results are consistent with the lack of major barriers to movement at the landscape scale, and consistent with the recognized ability of tortoises to move long distances.

Unfortunately, the dispersal ecology of this species is not well understood (Morafka 1994). However, individual tortoises have the potential to move long distances to forage or reproduce. Although few long forays (greater than 30 km) have been recorded (Edwards et al. 2004), long-distance dispersal events are difficult to detect using direct methods (Koenig et al. 1996, Nathan 2001). The long life span of tortoises, coupled with annual opportunities for reproduction during non-drought periods, allows individuals potentially to move longer distances over their reproductive lifetime (Edwards et al. 2004, Esque et al. unpublished data). This expanded period of influence and long generation time increases the potential for gene flow to homogenize populations over relatively short distances, causing isolation by distance to be a primary mechanism for any population differentiation.

Although the basal and hierarchical clusters identified by the Bayesian analysis were robust and informative, there are reasons to be cautious interpreting the results. Using STRUCTURE to determine the most likely number of populations is arbitrary and based on ad hoc criteria (Pritchard et al. 2000, Evanno et al. 2005, Pritchard et al. 2007). Although the ad hoc criteria coupled with diagnostics and biological context are thought to be relatively reliable, there is still the potential for misinterpretation (Pritchard et al. 2007). In particular, when allele frequencies differ only slightly between adjacent populations, the underlying model may produce results that are difficult to interpret because the algorithm is forced to search for distinct components whether distinct components exist or not.

In a previous study with simulated data, the model was able to detect the basal clusters in a contact zone migration model (Evanno et al. 2005). A hierarchical clustering analysis was effective in this scenario, which suggests that it is possible to make inferences from Bayesian models when demes are connected by localized gene flow (Evanno et al. 2005). However, the level of differentiation in these simulations was higher ( $F_{ST} = 0.16 - 0.43$ ; Evanno et al. 2005) than the levels detected in the Mojave desert tortoise ( $F_{ST} = 0.01 - 0.16$ ). The number of genetic markers used in our study, and their variability, gave us very high power to detect small differences in allele frequencies (Waples 1998a, Hedrick 1999, Ryman et al. 2006, Hedrick 2001) potentially magnifying differences of marginal biological significance. Thus, decisions concerning conservation actions should be fortified with ecological and behavioral information as well as genetic information.

Multimodality of the fitted models in STRUCTURE, and the varied effects of sampling design, also caused us to scrutinize the interpretation of our data. Low differentiation among populations, isolation by distance, or a combination may have caused the re-sampling algorithm to find more than one local maximum for simulations fixing the number of clusters to be more than four. However, multimodality did not occur when  $K$  was fixed to 2, 3, and 9, and also multimodality did not occur in the hierarchical analyses. Lack of multimodality in these instances provided support for scenarios of 2, 3, and 9 population clusters. Differences in sampling intensity also resulted in different numbers of genotype clusters and the boundaries to those clusters. Six clusters had the highest likelihood with reduced (and even) sample sizes, and this simulation indicated that the California cluster remained the same for each replicated analysis. However,

hierarchical analyses with reduced and even samples resulted in a partitioning of the California cluster in a biologically meaningful way by merging locations (e.g., Piute Valley) that was sampled more intensely in the full data set. It is difficult to reconcile the discrepancy between the analysis of the entire range using the reduced and even data set and the hierarchical analysis of basal clusters using the reduced and even datasets, except to acknowledge that sampling effort was not equal among all locations. Therefore, we support the use of caution with interpreting these analyses and stress the importance of sampling design for future assessments.

Finally, minor differences between the two types of Bayesian analysis require discussion. Both methods clearly identified broad scale population structure, including barriers to gene flow such as the New York and Providence Mountains. However, GENELAND identified different population boundaries for a fourth cluster in the Western Mojave Desert. Although this cluster was identified in subsequent hierarchical analyses with STRUCTURE, we assume that the addition of geographic information increased the likelihood that this cluster really exists. Minor irregularity in our sampling scheme (i.e., lack of sampling outside of Desert Wildlife Management Areas in the Western Mojave region) may have contributed to the detection of this area as a separate cluster. However, the model implemented in GENELAND appears to be robust to this type of irregularity in sampling design, though the detection of boundaries can be affected (Guillot et al. unpublished). Clearly, using multiple types of analyses to make informed inferences from population genetic data is a valuable approach (Manel et al. 2004, Rowe and Beebee 2007).

## Comparison to Other North American Tortoises

We detected low genetic differentiation among sampling locations in the range of the Mojave desert tortoise, which supported the major conclusions of other recent studies of *Gopherus agassizii* populations (Edwards et al. 2004, Murphy et al. 2007). A majority of genetic variation was captured within the populations, and isolation-by-distance was characterized as a major determinant of the pattern of differentiation.  $F_{ST}$  values for the desert tortoise (0.01 – 0.16) appear to be particularly low when compared to the gopher tortoise (*Gopherus polyphemus*), which inhabits sand hill, longleaf pine, and scrub ecosystems of the southeastern United States (Schwartz et al. 2005). Levels of genetic differentiation were notably higher in this species ( $\bar{F}_{ST} = 0.24 \pm 0.12$ ) (Schwartz et al. 2005). Further, geographic distance accounted for approximately 15% of the observed genetic variation for gopher tortoises (Schwartz unpublished). In striking contrast, 65% of observed genetic variation is explained by geographic distance for the Mojave desert tortoise (this study and Murphy et al. 2007). Gopher tortoises are known to have limited migratory ability and very small home ranges (McRae et al. 1981, Diemer 1992, Eubanks et al. 2003, Schwartz et al. 2005), and existing gopher tortoise populations are restricted mainly to protected parkland due to extensive habitat destruction and fragmentation (Kautz 1993, Schwartz et al. 2005). Behavioral differences, and naturally limited migration, could elucidate the different patterns of genetic differentiation.

Although Sonoran desert tortoises also exhibit a pattern of isolation by distance, only 30% of the observed variation is explained by distance (Edwards et al. 2004). Differential use of available habitat may account for the disparity in the amount of

genetic variance explained between Mojave and Sonoran desert tortoise populations (Van Devender 2002). Sonoran desert tortoises tend to inhabit rocky foothills, which are more naturally fragmented than are the bajadas typically occupied by Mojave desert tortoises (Van Devender 2002).

Despite a similar global  $F_{ST}$  value (0.06) and similar patterns of differentiation (isolation-by-distance and majority of variation occurring within populations), conclusions from our study differ very much from another recent assessment of the Mojave desert tortoise using microsatellites (Murphy et al. 2007). The genotype clusters identified by Murphy et al. (2007) align closely with the current six recovery units described in the original Recovery Plan (USFWS 1994); however, the authors also detected additional sub-structuring within the Western Mojave Recovery Unit (Western, Southern, Central Mojave units). Therefore, they suggested that the original Western Mojave Recovery Unit should be further bisected into three units, increasing the total number of recovery units to eight. The authors did not recommend any other changes to recovery unit boundaries.

The boundaries of genetic units detected in our study differed from Murphy et al. 2007 and from the original recovery units (USFWS 1994). We identified seven genotype clusters for the Mojave desert tortoise that reflect isolation-by-distance coupled with geographic barriers preventing localized gene flow. The main boundary differences between the two studies exist in the northern portion of the range, where we detected additional genetic variation requiring further delineation of the Northeastern Mojave Recovery Unit. The population in the Upper Virgin River Recovery Unit has been cited as ecologically and behaviorally dissimilar from other populations, which was supported

by extreme allele frequency differences (Murphy et al. 2007). However, we determined that the tortoises in the region surrounding St. George, UT consistently, and strongly, cluster with adjacent locations in the Beaver Dam Slope, Mormon Mesa, and Gold Butte. Additionally, we detected a boundary along the New York, Providence, and McCullough Mountains, which separates a portion of the Eastern Mojave Recovery Unit and the Northern Colorado Recovery Unit. The locations west of these mountain ranges grouped with a genotype cluster not previously recognized (Amargosa cluster). Finally, we did not detect any further sub-structuring in the Western Mojave Recovery Unit.

Differences between these two studies in the delineating population boundaries can be attributed to sampling design. Careful investigation of population genetic structure requires comprehensive, and thorough, sampling of potential populations (Evanno et al. 2005). Furthermore, population structure should be inferred from random sampling across the landscape (Manel et al. 2003, Guillot et al. 2005a). We favored spreading our sampling effort across more populations, even if we could not get the desired sample size in each area, over sampling to get large sample sizes in fewer locations (Pons and Chaouche 1995). Additionally, we selected microsatellite markers with several alleles improved our ability to estimate genetic parameters (Lowe et al. 2004, Ryman et al 2006).

Previously, genetic research for the Mojave desert tortoise was conducted in conjunction with other studies, or was limited in geographic scope, which constrained sampling design and the ability to detect population boundaries (Lamb et al. 1989, Britten et al. 1997, Berry et al. 2002, Tracy et al. 2004). As a result, additional research with the expressed intent of identifying genetic units was recommended (Berry et al. 2002, Tracy

et al. 2004). Although Murphy et al. (2007) sampled representative individuals from each of the six original recovery units (USFWS 1994), a majority of the sampling (73%) was confined to the Western Mojave Recovery Unit, with 30% of the total samples collected within a single 60 km area (Marine Corps Air Ground Combat Center, Twentynine Palms, CA). Murphy et al. used samples collected over a ten-year period from previous studies mainly related to disease detection within Desert Wildlife Management Areas. As a result, sampling was opportunistic, and many samples were collected on plots of varying size, but usually only a few square kilometers in area.

Unfortunately, this sampling design is not ideal to evaluate spatial population structure, as it may not capture spatial variation (Storfer et al. 2007). Sampling many individuals in close proximity may increase the probability of sampling very closely related individuals within demes, which violates assumptions of the Bayesian analyses, and can lead to an overestimation of the number of distinct genotype clusters (Pritchard et al. 2007). Our work also shows that unequal sampling intensity increases the potential to overestimate the number of genotype clusters even if the samples are not dominated by sampling demes. This result has been discussed previously (McRae et al. 2005). Therefore, the intensive sampling in the Western Mojave recovery unit (Murphy et al. 2007) likely created spurious, additional clusters in the Bayesian analyses. In our study, pair-wise  $F_{ST}$  values among locations in these regions were  $< 0.02$  and were not statistically significant. Although the sampling design for our study was not completely randomized or inclusive, we accounted for unequal sampling intensity and adjusted the interpretation of any potential genotype clusters.

Incomplete sampling across portions of the range (e.g., Nevada) also potentially caused spurious results in the study by Murphy et al (2007). They noted that the Upper Virgin River recovery unit and the Northeastern Mojave recovery unit were the most differentiated groups and were more isolated than the other sampled groups. However, they sampled no locations in Nevada, and that likely artificially led to the appearance of great differentiation in the northern-most sample site. Including locations in Nevada in our study revealed a gradient of small genetic differentiation. Thus, our analyses do not support great differentiation in the northern-most locations of the desert tortoise.

Recent simulations have addressed the potential problem of not sampling “ghost populations”, and the effect of this inadequate sampling on estimating migration rates (Beerli 2004, Slatkin 2005). The interdependence of populations is complicated, and although it may not be completely necessary to sample all populations, high levels of migration from unsampled populations impact estimates of migration rates (Beerli 2004). In a similar way, the allele frequencies used to infer population structure likely will be different with the absence of known locations of the species. The comparison of the Murphy et al. study to ours underscores the potential for markedly different interpretations of population genetic data and analyses when study design and sampling differ. Although the microsatellite markers used in each of these two studies were not identical, multiple metrics, including F-statistics, analysis of molecular variance, and Mantel tests, were strikingly similar. Despite these similarities, the inferences in each study were markedly different. Therefore, we contend that highly skewed sampling intensity coupled with lack of sampling in a portion of the core distribution of a species prohibited Murphy et al. from making accurate inferences from population genetic

assessments at the landscape scale. The shift from population-based analyses to individual-based analyses in population genetics research requires a change in the design of studies and how samples are collected. If sampling is not modified to reflect these new approaches to data analysis, inferences and conclusions drawn from data may be misleading.

### Recommendations for Conservation Practices

The life history traits of the desert tortoise (i.e., very long life span and generation time) framed the temporal scale of our investigation. Therefore, we described population structure that was shaped by many generations (and therefore many hundreds of years) prior to the recent surge of anthropogenic impacts on the southwestern United States. As such, our analyses cannot detect potential isolation due to recent urbanization of the Mojave and Colorado Deserts. Indeed, the long generation time of desert tortoise makes it unlikely that our analyses could assess the impacts of anthropogenic relocations of tortoises that have occurred within the last several decades. Fortunately, this perspective allows us to infer the spatial genetic structure of tortoise populations prior to severe human influence, which can offer direction for the maintenance of natural (or at least semi-natural) population dynamics. We are able to make several recommendations to revise conservation strategies for the Mojave desert tortoise (see below).

## Delineation of Conservation Units

Recovery planning was initiated subsequent to the federal listing of the Mojave Desert tortoise in 1990 under the Endangered Species Act of 1973 (55 FR 12178, April 2, 1990). This planning included crafting a recovery plan and delineating biologically distinct populations within the range of the Mojave desert tortoise as a means to facilitate management and preserve intraspecific diversity. Original authors of the Recovery Plan used the concept of the evolutionarily significant unit (ESU; Ryder 1986, Waples 1991, Mortiz 1994) to describe geographic units (termed Recovery Units), which were deemed important to conserve adequately the diversity of the listed entity (USFWS 1994). The concept of the ESU has remained widely used and debated in the academic literature (Pennock and Dimmick 1997, Waples 1998b, Paetkau 1999, Taylor and Dizen 1999, Crandall et al. 2000, Green 2005). However, the management unit (Moritz 1994, Paetkau 1999, Palsboll et al. 2006) may be a more applicable conservation unit to diagnose entities within the Mojave desert tortoise. Management units (MU), which can be defined as populations with independent dynamics, are typically considered as less isolated than ESUs and are useful for identifying local conservation and monitoring (Palsboll et al. 2006). Although these terms and the discussion surrounding them have merit and are necessary to establish a biological basis for delineations, the MU and the ESU are not policy terms or legally binding.

Currently, the Mojave Desert tortoise is listed as a distinct population segment (DPS) (55 FR 12178, April 2, 1990), which is a legal entity under the ESA (ESA, Section 4). As such, its status can be changed independently of other DPS or species/subspecies. The policy that defined a DPS originally pertained to salmonids and was based on the

ESU (NMFS 1991, Waples 1991, Waples 1995). Revisions to this policy further clarify the definition and provide guidelines for how a DPS can be listed, delisted, or reclassified using the criteria of discreteness, significance, and conservation status for all vertebrate taxa (USFWS and NOAA 1996, Rosen 2007). Genetic data are relevant to satisfying the criteria for both discreteness and significance; however other morphological, ecological, and behavioral evidence are also applicable (USFWS and NOAA 1996).

Within the scope of recovery planning, the recovery unit remains the workhorse of conservation unit delineations for many listed species. Delineated recovery units contain features that ensure the recovery and long-term viability of the listed entity (NMFS 2006). In contrast to the DPS, the recovery unit is described only within a recovery plan and is not formally listed through the ESA. Therefore, recovery units are not protected individually by the Act, nor can their status be changed separately from other units. Although these units may be treated as individual management units, the population(s) contained in each recovery unit within the listed entity must exhibit signs of recovery before it can be removed from the endangered species list (NMFS 2006). Therefore, the subsequent discussion of conservation units for the desert tortoise refers to diagnosing management units (generally) and recovery units (specifically). If further delineations of distinct population segments for the desert tortoise were considered by the USFWS in the future, these data would be applicable to that decision as well.

We recommend that the boundaries of conservation units for the Mojave desert tortoise be revised to reflect fine-scale genetic structure identified in this investigation. Although we detected only low-to-moderate levels of genetic differentiation range wide, these delineations reflect differentiation derived from natural levels of gene flow in a

system driven by isolation-by-distance. Additionally, localized dispersal and natural barriers have prevented homogenization of these populations over many generations. Across the range, we recommend delineating seven conservation units. However, simply rejecting panmixia may not be sufficient for the delineation of conservation units, and some types of population structure (e.g. isolation-by-distance) do not lend themselves to definitive boundaries (Palsboll et al. 2006). The recommended changes to delineations should be treated as a new hypothesis that is tested with additional genetic, demographic, ecological, and behavioral data, including estimates of dispersal rates among proposed genotype clusters, and biotic interactions (e.g., host-pathogen relationships) within the ecologically different areas of the range of the Mojave desert tortoise (Palsboll et al. 2006).

Delineating conservation units (or recovery units) should not be based solely on population genetics (Paetkau 1999, Taylor and Dizon 1999, Green 2005). Genotype clusters described with neutral markers provide an excellent starting point for delineating conservation units (Palsboll et al. 2006); however, these data and analyses do not reflect other unique ecological, behavioral, and morphological characteristics or conservation status (Green 2005). In the case of the desert tortoise, the temporal scale of analyses prevents us from detecting any population genetic signatures from recent fragmentation of habitat, anthropogenic influences on habitat or populations, or population declines. However, known threats to population persistence differ dramatically across the range and population declines are spatially heterogeneous (Tracy et al. 2004). Conservation units should not only reflect genetic considerations and conservation status, but also ecological considerations broadly speaking to include landscape differences as well as

local differences in geography, vegetation, and physiognomy. Diversity of food and shelter resources must be captured in conservation units to ensure temporal and spatial abilities to meet the needs of individual tortoises as a way to bolster the viability of populations and avoid periodic natural threats to persistence including climate change. Therefore, these units must capture unique habitats and unique ecological interactions, as well as variability in behavior and life history traits.

Our data provide no direct link between genetic variation and traits that could provide a selective advantage across the habitat types that exist throughout the range of the Mojave desert tortoise. However, the genotype clusters that we have identified encompass variation in life history characteristics, activity patterns, behavioral traits, and habitat types. Each of the recommended conservation units contains a portion of the regional variation in survival rates, causes of mortality, and reproductive output (Nagy and Medica 1986, Germano 1994a, Peterson 1994, Peterson 1996, Henen et al. 1998, Mueller et al. 1998, Lovich et al. 1999, Tracy et al. 2004). For example, tortoise reproduction varies across a longitudinal gradient; tortoises in the western Mojave Desert (which typically receives mostly summer rains) produce relatively larger eggs, produce fewer eggs overall, and lay their second clutches later than do tortoises in the adjacent eastern Mojave Desert (Wallis et al. 1999). Behaviorally, western Mojave tortoises are much less active during summer than are tortoises in other regions (Marlow 1979, Nagy and Medica 1986). Extremely winter-dominant rainfall and resultant effects on the vegetation community, as well as its position on the western end of the distribution, contribute to the significance of this conservation unit (USFWS 1994).

The tortoises located near St. George, Utah represent the northern-most extent of the distribution of this species. The genetic data presented here do not support the delineations of the current Upper Virgin River recovery unit to be entirely in Utah; however, other unique features of these tortoises warrant additional protection. Desert tortoises in this regions experience long, cold winters (about 100 freezing days) and mild summers, during which the tortoises are continually active (Woodbury and Hardy 1948). Here tortoises live in a complex topography consisting of canyons, mesas, sand dunes, and sandstone outcrops where the vegetation is a transitional mixture of sagebrush scrub, creosote bush scrub, blackbrush scrub, and a psammophytic community. Desert tortoises use sandstone and lava caves instead of tortoise-constructed burrows, travel to sand dunes for oviposition, and use still other habitats for foraging. Often, two or more desert tortoises use the same burrow or cave (Woodbury and Hardy 1948, Esque 1994), which is less common in the southern and western portions of the range. Clearly, these tortoises have conservation potential despite the lack of supporting genetic differentiation. However, it seems prudent not to manage this tortoise population in complete isolation due to the evidence for historic gene flow with adjacent locations.

#### Maintenance of Population Structure

Severe anthropogenic impacts to desert tortoise habitat, including fragmentation due to highways, has only occurred in the past five to six decades (Lovich and Bainbridge 1999, Hunter et al. 2003). Desert tortoises have a relatively long generation time (estimated as more than 25 years; USFWS 1994). The age of first reproduction is determined by body size in females (sexual maturity occurs approximately at minimum

size of 180 mm; Turner et al. 1986, Germano 1994a) and individual growth rate varies in relation to available forage and drought (Germano 1994b, Mueller et al. 1998). Assuming a 25-year generation time, a conservative estimate of four generations may have occurred in the past century. Any potential genetic signature of habitat fragmentation and subsequent reduction in gene flow should not be observable yet. Further, fencing major roadways and public lands has made movement among critical habitat effectively impossible (Edwards et al. 2004).

We speculate that urban development has severely disrupted the natural migration and dispersal patterns of the desert tortoise, and that it is not possible to detect these disruptions due to the long temporal scale over which population dynamics occur in this species. The low levels of genetic differentiation that we have detected suggest that, until recently, tortoise populations were well connected. Recent habitat suitability models supported our hypothesis of past population connectivity (Thomas et al. in review). In a future population genetic assessment, researchers may have the power to detect the isolation of tortoise populations.

Desert tortoises have been translocated among locations in recent history (a) for management purposes (Murphy et al. 2007), (b) for research (Nussear 2004, Field et al. 2007), and prior to the listing of the species, (c) tortoises were removed from the wild as part of the pet trade (Murphy et al. 2007). Captive releases of individuals have been recorded periodically. These translocations have the potential to interfere with the ability to detect a population genetic signature (Murphy et al. 2007). However, many of these translocations were poorly documented, and there is scant information beyond anecdotes to suggest that these translocations resulted in successful reproduction in the new

locations. Early translocations were often executed when the seasonal temperatures were inhospitable for tortoises, and shelter was not provided for the translocated tortoises, which generally resulted in the translocated tortoises dying (Cook et al. 1978, Cook 1983, Nussear 2004, Field et al. 2007). Indeed, many early translocations were not successful because tortoises were exposed to lethal thermal environments or novel predators (Cook 1983, Nussear 2004, Field et al. 2007). However, translocations have been successful (i.e., high survivorship and typical egg-laying behavior) when they occurred during the spring when seasonal temperatures were below lethal limits and forage was available (Nussear 2004).

The only evidence of potentially successful translocations threatening to taint our data set is in the Red Cliffs Desert Reserve near St. George, UT. Reportedly, individuals had been moved from California to the St. George area, however, we were able to discern likely translocated individuals, and they all had a genetic signature from south Las Vegas Valley, not to California. Further, all individuals in our analyses were assigned to their original cluster, or to an adjacent cluster. This is consistent with isolation-by-distance and historically high levels of gene flow. The possibility that translocations have augmented the signal of gene flow among clusters does exist; however, there is limited evidence that this factor warrants scrutiny. Similar to other anthropogenic impacts, translocations have only occurred in recent history. Unless the actual translocated animals were sampled in a population genetic assessment, the long generation time of these animals would prevent any potential progeny showing up in a sample of adult tortoises, and thus would not be detected in our study or other recent studies.

Future management should take into account our analyses using genetic markers. If adult individuals are translocated to supplement declining populations, or they are cleared from habitat that is slated for urban development, care should be taken to transport individuals to within their genotype cluster. This consideration should complement other recommendations from previous studies (Nussear 2004, Field et al. 2007). Additionally, managers should avoid transporting tortoises across the potential boundaries to gene flow identified here. Head-starting populations with young recruits is a potential management action that may be implemented to augment poor recruitment in some locations (Iverson 1991, Congdon et al. 1993, but see Heppell et al. 1996). However, in any head starting program, mated adults should originate from the same genotype cluster, and offspring should be released in that cluster to maintain current levels of genetic diversity and avoid excessive outbreeding (Frankham et al. 2002). Although rules of thumb could be used to prevent negative consequences on average, extensive research using mating experiments would be required to determine the actual fitness consequences of a breeding program.

Although we can infer from summary F-statistics that moderate to high levels of gene flow occurred among adjacent tortoise subpopulations, estimates of migration rates from F-statistics are not reliable (Whitlock and McCauley 1999). Therefore, additional genetic analyses and field studies would complement what is known about movement from our genetic data. More information about dispersal would be valuable and would have direct management implications. In the past, long-distance migration may have been critical to the persistence of desert tortoise populations. Catastrophic die-offs have been documented periodically (Peterson 1994), and recolonization from adjacent valleys may

be necessary to ensure population viability. This rescue effect (Brown and Kodric-Brown 1977, Hanski and Gilpin 1997) could have profound implications across the temporal scale in which tortoise populations operate.

Translocations or other management actions, such as the addition of culverts under highways to allow natural movement, may be necessary to improve connectivity and maintain historic levels of gene flow across cluster boundaries that have been eliminated by human actions. Although the effectiveness of culverts as habitat linkages has been demonstrated for other species (Clevenger et al. 2001), limited research has been conducted on how well culverts facilitate tortoise movement (Fusari 1985, Ruby et al. 1994, Boarman et al. 1996). Results from this research are promising, suggesting that culverts (if large enough) have the potential to be an effective method for maintaining connectivity (Ruby et al. 1994). Fencing of major roadways has certainly decreased mortality of adult tortoises (Boarman et al. 1996), but this management action has fragmented habitat (von Seckendorff Hoff and Marlow 2002), and halted potential gene flow within and among tortoise populations (Ruby et al. 1994, Edwards et al. 2004). Facilitating movement among populations may be a critical component to management strategies for this threatened distinct population segment.

## **CONCLUSIONS**

Isolation-by-distance and low levels of genetic differentiation characterize population structure in the Mojave desert tortoise. Using individual-based Bayesian assignment tests, we identified hierarchical structuring in this threatened distinct

population segment. The three basal clusters corresponded to mtDNA haplotypes, and we detected additional spatial structure within the basal clusters. Uneven sample sizes in some areas appear to have created spurious clusters; however, seven of the finer scale clusters were robust to our sampling scheme. Therefore, we recommend that the boundaries of conservation units for the Mojave desert tortoise be changed to account for these new analyses. Our recommended boundaries do not align with recommendations from other genetic studies of the Mojave desert tortoise using microsatellites; however, the noticeable differences in sampling design between the studies account for these differences.

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# TABLES

Table 1. Sampling locations for Mojave desert tortoises (N = 748) based on geography (including the state and abbreviation for the site), the number of individuals from each location (N), and how samples were collected (STS = systematic transect sampling; LDS = line distance sampling (random)). Each site is associated with a desert tortoise Recovery Unit; however, these delineations are only approximate due to sampling sites crossing Recovery Unit boundaries.

Recovery Unit	Sampling location	Abr.	State	Sample collection	N
Upper Virgin River	Red Cliffs Desert Reserve	RC	UT	STS	33
Northeastern Mojave	Beaver Dam Slope	BD	UT, NV	LDS, STS	12
	Mormon Mesa	MM	NV	LDS, STS	43
	Gold Butte-Pakoon Basin	GB	NV, AZ	LDS, STS	17
	Coyote Springs	CS	NV	LDS, STS	26
	Muddy Mountains	MD	NV	STS	30
	Northeast Las Vegas Valley	NEL	NV	STS	20
	Northwest Las Vegas Valley	NWL	NV	STS	21
	Pahrump Valley	PA	NV	STS	27
	Amargosa Desert, Oasis Valley, Greenwater Valley	AM	NV, CA	STS	18
	Southwest Las Vegas Valley	SWL	NV	STS	28
	South I-15 Corridor (Goodsprings, Jean Dry Lake, Sloan)	SI	NV	STS	29
	Southeast Las Vegas Valley (River Mountains)	SEL	NV	STS	12
	Eldorado Valley	EL	NV	LDS, STS	49
	Piute Valley	PI	NV	LDS, STS	80
	Ivanpah Valley	IV	CA	LDS, STS	16
Eastern Mojave	Shadow Valley	SV	CA	STS	17
	East Providence Mountains	EP	CA	LDS, STS	38
	West Providence Mountains	WP	CA	LDS, STS	14
	Chemehuevi DWMA	CM	CA	LDS	59
Northern Colorado					
Eastern Colorado	Chuckwalla DWMA	CK	CA	LDS	56
Eastern Colorado	Pinto Mountains	PM	CA	LDS	
Colorado/West Mojave	DWMA/Joshua Tree NP				25
Western Mojave	Ord-Rodman DWMA	OR	CA	LDS	14
	Superior-Cronese DWMA	SC	CA	LDS, STS	45
	Fremont-Kramer DWMA	FK	CA	LDS	19

Table 2. Mean gene diversity ( $\pm 1$  standard deviation), mean allelic richness ( $\pm 1$  standard deviation), and  $F_{IS}$  (significant values after Bonferroni correction of  $P < 0.0001$  are in bold) for each sampling location for Mojave desert tortoises.

Location	Gene diversity ( $\pm$ SD)		Allelic Richness ( $\pm$ SD)		$F_{IS}$
RC	0.712	0.207	6.413	2.668	0.072
BD	0.656	0.263	5.568	2.644	0.079
MM	0.687	0.238	6.114	2.737	0.011
GB	0.643	0.279	5.593	2.624	<b>0.142</b>
MD	0.750	0.241	7.357	3.380	<b>0.075</b>
CS	0.723	0.235	7.078	3.445	0.061
NEL	0.744	0.267	7.416	3.423	-0.003
NWL	0.756	0.215	7.589	3.197	0.061
AM	0.742	0.215	6.999	3.156	0.036
PA	0.765	0.215	7.499	3.199	0.059
SH	0.768	0.188	7.253	3.149	0.051
IV	0.788	0.206	7.655	3.182	0.039
WP	0.780	0.195	7.970	3.515	0.027
SI	0.786	0.169	7.442	3.022	0.035
SWL	0.780	0.209	7.993	3.816	0.038
SEL	0.799	0.173	7.606	3.105	0.047
EL	0.780	0.198	7.406	3.041	<b>0.069</b>
PI	0.779	0.209	7.920	3.172	<b>0.061</b>
CM	0.739	0.232	7.517	3.345	<b>0.058</b>
EP	0.746	0.222	7.556	3.204	<b>0.06</b>
CK	0.721	0.253	7.078	3.359	0.044
PM	0.724	0.257	7.288	3.574	0.056
OR	0.737	0.239	7.048	3.392	0.072
SC	0.725	0.234	7.024	3.423	0.026
FK	0.721	0.237	6.916	3.047	0.098
Overall	0.742	0.040	8.352	3.354	0.053

Table 3. Mean  $\ln P(D)$  ( $\pm 1$  standard deviation) and the second order rate of change calculations for  $\Delta K$  when  $K$  was fixed to  $K = 1$  through  $K = 10$  in STRUCTURE.

K	Mean $\ln P(D)$	$\pm$ SD $\ln P(D)$	Mean $L'(K)$	Mean $L''(K)$	$\Delta K$	
1	-64113.	8.37				
2	-60625.	1.39	3487.1	2572.35	<b>1845.73</b>	0.187
3	-59918.	2.73	707.06	557.65	<b>204.28</b>	0.078
4	-59769.	578.13	149.41	523.2	0.91	0.054
5	-59242.	4.33	527.45	309.63	71.46	0.049
6	-59011	72.55	230.66	144.23	1.99	0.046
7	-58776	77.10	234.65	158.84	2.06	0.045
8	-58595	20.94	180.75	83.62	3.99	0.043
9	-58482	6.79	113.59	98.23	14.46	0.041
10	-58461	20.44	21.94			0.041

Table 4. Mean  $\ln P(D)$  and  $\Delta K$  for each of the three basal clusters in STRUCTURE.

These additional analyses were used to detect hierarchical clustering within the Mojave population of the desert tortoise. \* indicates the  $K$  with the highest mean  $\ln P(D)$  and  $\Delta K$

Basal cluster	$K$	Mean $\ln P(D)$	$\Delta K$	Description of hierarchical clusters
<b>Northern Mojave</b>	1	-13456.4		
	<b>2*</b>	<b>-13191.9</b>	<b>16.9</b>	The Northern Mojave was divided into two clusters. Cluster 1 (Virgin River) contained RC, BD, MM. Cluster 2 (Muddy Mountains) contained GB, MD, CS, and NEL.
	3	-13219.1	9.0	
	4	-13359.0	7.3	
	5	-14842.2	4.2	
	6	-14295.2	2.6	
	7	-14497.5	1.7	
<b>Las Vegas</b>	1	-17997.5		
	2	-17925.7	1.8	The Las Vegas cluster was divided into three clusters. Cluster 1 (Amargosa Desert) contained AM, PA, and SH. Cluster 2 (South Las Vegas) contained NWL, IV, SI, SWL. Cluster 3 (Eldorado) contained El and SEL.
	<b>3*</b>	<b>-17807.7</b>	<b>43.1</b>	
	4	-18187.4	3.2	
	5	-18006.1	2.9	
	6	-18235.4	2.5	
	7	-18905.2	2.4	
	8	-19387.3	1.6	
	9	-19454.3	2.1	
	10	-19686.9	1.4	
<b>California</b>	1	-28618.9		The California cluster was divided into four additional clusters. Cluster 1 (Piute Valley) contained PI and WP. Cluster 2 (Northern Colorado) contained CM and EP. Cluster 3 (Eastern Colorado) contained CK and PM. Cluster 4 (Western Mojave) contained OR, SC, and FK.
	2	-28184.7	44.7	
	3	-27885.3	38.1	
	<b>4*</b>	<b>-27683.7</b>	<b>55.7</b>	
	5	-27753.1	19.1	
	6	-28166.4	2.0	
	7	-28822.3	2.2	
	8	-28965.1	1.7	

Table 5. Log of the posterior density of the model for 10 independent runs of GENELAND. The modal  $K$  is the optimal number of genotype clusters for desert tortoises across 500,000 iterations of the model.

Run number	Log Posterior Density	Modal $K$
1	-57516	3
2	-57954	4
3	-58033	4
4	-57934	4
5	-57643	3
6	-57960	4
7	-57963	4
8	-57958	4
9	-57950	4
10	-57954	4

Table 6. Analysis of molecular variance for three genotype clusters as determined via STRUCTURE. The percentage of variation explained by each source was similar for  $K = 3$ ,  $K = 4$ ,  $K = 7$ , and  $K = 9$ . \* significance at  $P < 0.05$  when compared to 1023 permutations of the data.

<b>Source of Variation</b>	<b>df</b>	<b>Sum of Squares</b>	<b>Variance components</b>	<b>Percentage variation</b>
Among groups	2	417.22	0.389	4.94*
Among populations within groups	22	445.61	0.218	2.78*
Among individuals within populations	723	5468.89	0.300	3.82*
Within populations	748	5208.5	6.96	88.46*
Total	1495	11540.22	7.87	

Table 7. Pair-wise  $F_{ST}$  values for the nine inferred genotype clusters of Mojave desert tortoises. All values were significant using an adjusted P value ( $P < 0.00139$ ) after 720 permutations. Cluster IDs are: VR = Virgin River; MD = Muddy Mountains; AM = Amargosa Desert; SLV = South Las Vegas Valley; EL = Eldorado Valley; PI = Piute Valley; NCO = Northern Colorado Desert; ECO = Eastern Colorado Desert; WM = Western Mojave Desert.

	<b>VR</b>	<b>MD</b>	<b>AM</b>	<b>SLV</b>	<b>EL</b>	<b>PI</b>	<b>NCO</b>	<b>ECO</b>	<b>WM</b>
<b>VR</b>	-								
<b>MD</b>	0.025	-							
<b>AM</b>	0.044	0.016	-						
<b>SLV</b>	0.048	0.019	0.012	-					
<b>EL</b>	0.067	0.038	0.023	0.014	-				
<b>PI</b>	0.087	0.057	0.041	0.029	0.020	-			
<b>NCO</b>	0.114	0.082	0.062	0.051	0.040	0.011	-		
<b>ECO</b>	0.132	0.097	0.086	0.066	0.057	0.028	0.026	-	
<b>WM</b>	0.125	0.082	0.071	0.057	0.052	0.032	0.032	0.031	-

Table 8. Number of assignments of desert tortoises to one of the nine inferred genotype clusters. Cluster IDs are: VR = Virgin River; MD = Muddy Mountains; AM = Amargosa Desert; SLV = South Las Vegas Valley; EL = Eldorado Valley; PI = Piute Valley; NCO = Northern Colorado Desert; ECO = Eastern Colorado Desert; WM = Western Mojave Desert.

	<b>VR</b>	<b>MD</b>	<b>AM</b>	<b>SLV</b>	<b>EL</b>	<b>PI</b>	<b>NCO</b>	<b>ECO</b>	<b>WM</b>	<b>Assigned (%)</b>
<b>VR</b>	92	9	0	2	2	0	0	0	0	87.6
<b>MD</b>	10	58	3	4	1	0	0	0	0	76.3
<b>AM</b>	0	7	57	12	3	4	0	0	0	68.7
<b>SLV</b>	1	3	14	55	11	1	0	0	0	64.7
<b>EL</b>	0	0	2	4	40	3	0	0	0	81.6
<b>PI</b>	0	0	1	1	7	59	8	3	1	73.8
<b>NCO</b>	0	0	1	3	5	7	78	9	8	70.3
<b>ECO</b>	0	0	0	0	0	2	5	68	5	85.0
<b>WM</b>	0	0	0	0	0	0	2	4	73	92.4

## FIGURE LEGENDS

Figure 1. Map of subpopulations for the Mojave desert tortoise. Each point indicates each location where a blood sample was collected. The marker type indicates the three basal clusters (square = Northern Mojave, circle = Las Vegas, diamond = California). The color of the marker further indicates sub-structuring (Virgin River = red, Muddy Mountains = light blue, Amargosa Desert = orange, South Las Vegas = dark blue, Eldorado Valley = teal, Piute Valley = purple, Northern Colorado = green, Eastern Colorado = yellow, Western Mojave = pink).

Figures 2. Results from Program STRUCTURE using 20 microsatellites and 748 individuals from 25 sampling locations. (a) Number of genotype clusters based the mean  $\ln P(D)$  (red circles) and  $\Delta K$  (blue squares and dotted line) using  $K = 1$  and  $K = 2$  as a starting point for 10 separate MCMC chains with fixed  $K$  from  $K = 1$  to  $K = 10$ ; (b) Representative bar plot for  $K = 3$ . Bar plots indicate proportional membership of each individual to one (or more than one) genotype cluster.

Figure 3. Nine genotype clusters identified with Program STRUCTURE. Mean proportional membership ( $\pm 1$  standard deviation) to nine genotype clusters.

Figure 4. Mean proportional membership ( $\pm 1$  standard deviation) to six genotype clusters identified using STRUCTURE when each sampling location has  $n \leq 30$ .

Figure 5. Representative bar plots from STRUCTURE for hierarchical structuring when each sampling location has  $n \leq 30$ . (a) Las Vegas Cluster, (b) California cluster. The Las Vegas cluster was divided into two clusters. Cluster 1 (Amargosa Desert) contained AM, PA, and SH. Cluster 2 (South Las Vegas) contained NWL, IV, SI, SWL, SEL, and EL. The California cluster was divided into three

clusters. Cluster 1 (Northern Colorado) contained PI, WP, CM and EP. Cluster 2 (Eastern Colorado) contained CK and PM. Cluster 3 (Western Mojave) contained OR, SC, and FK.

Figure 6. Map of posterior probability of membership to four genotype clusters identified using GENELAND. Each black point corresponds to a desert tortoise location. Lighter shading represents higher probability of membership. (a) Western Mojave Desert cluster (WM); (b) Virgin River cluster (VR); (c) Las Vegas cluster (LV); and (d) Colorado Desert cluster (CO).

Figure 7. Isolation by distance across sampling locations of the desert tortoise in the Mojave Desert. Points represent comparisons of genetic distance ( $F_{ST}/1-F_{ST}$ ) as a function of geographic distance between centroids for sampling locations ( $R^2 = 0.6783$ ,  $P < 0.0001$ ).

**FIGURES**

Figure 1.

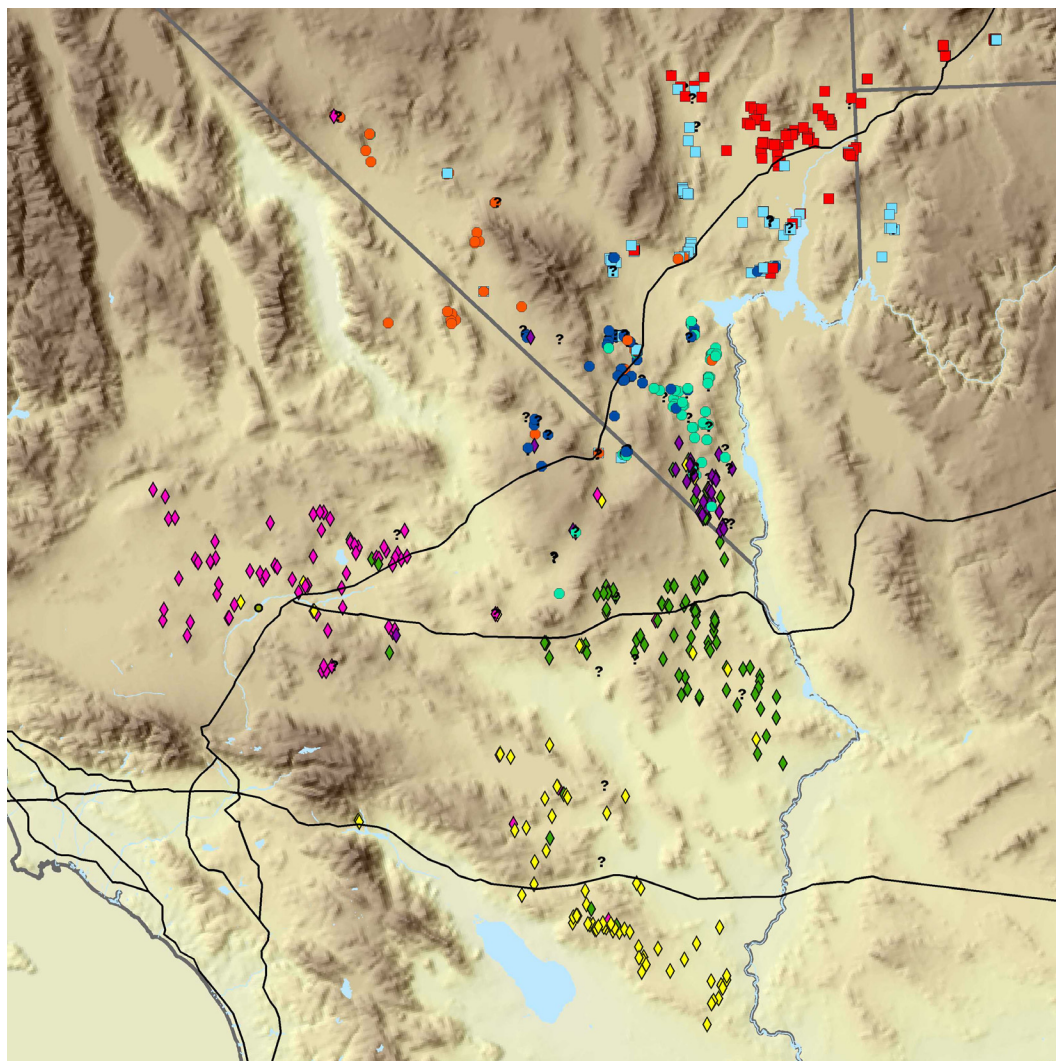


Figure 2a.

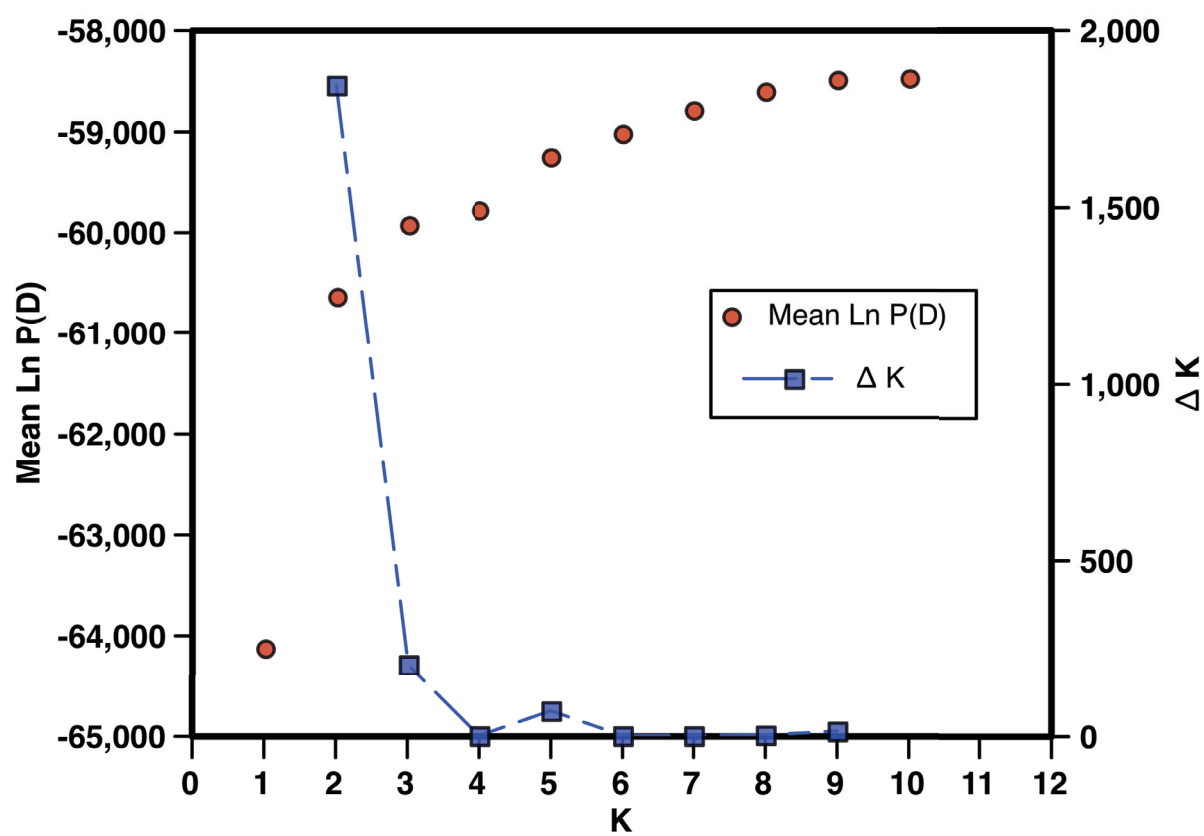


Figure 2b.

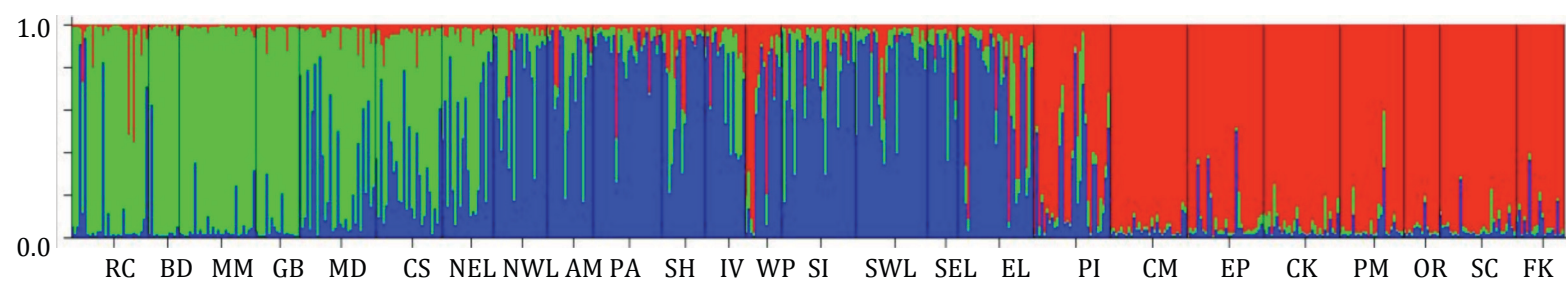


Figure 3.

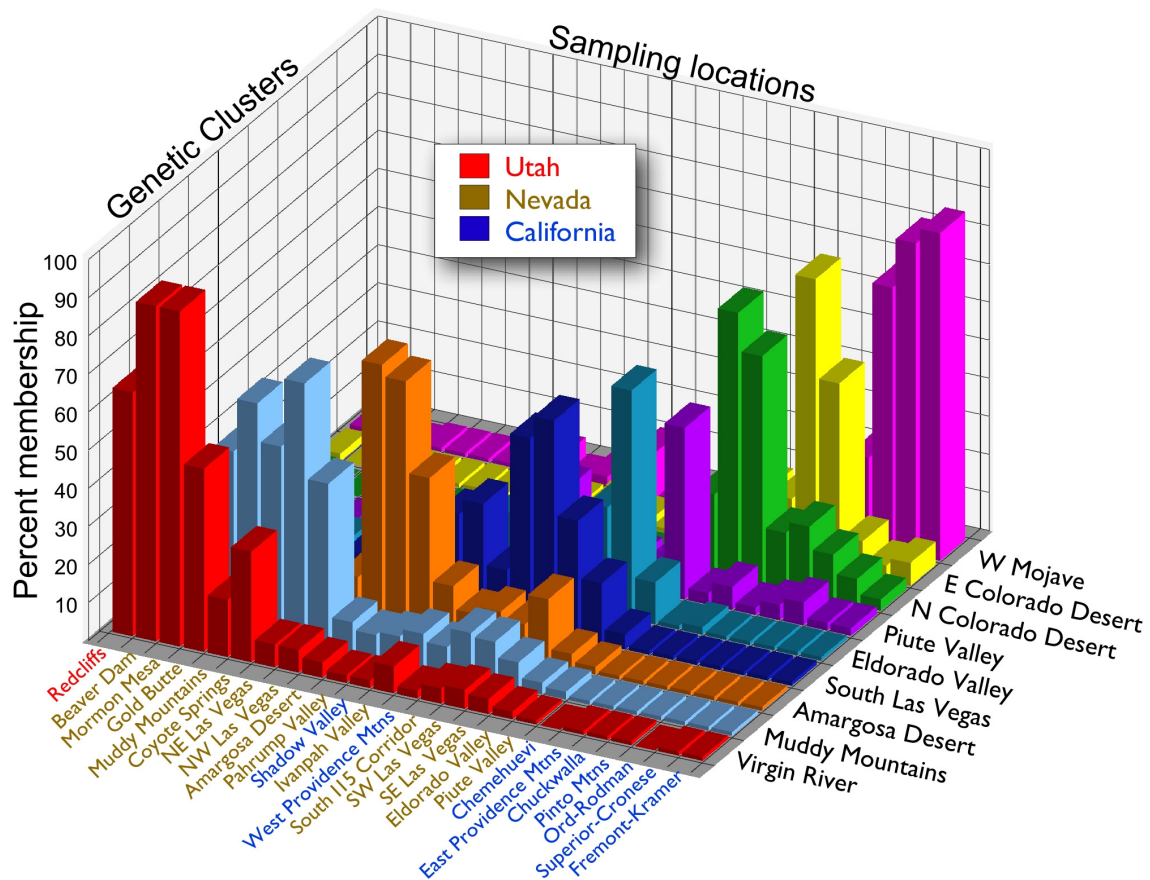


Figure 4.

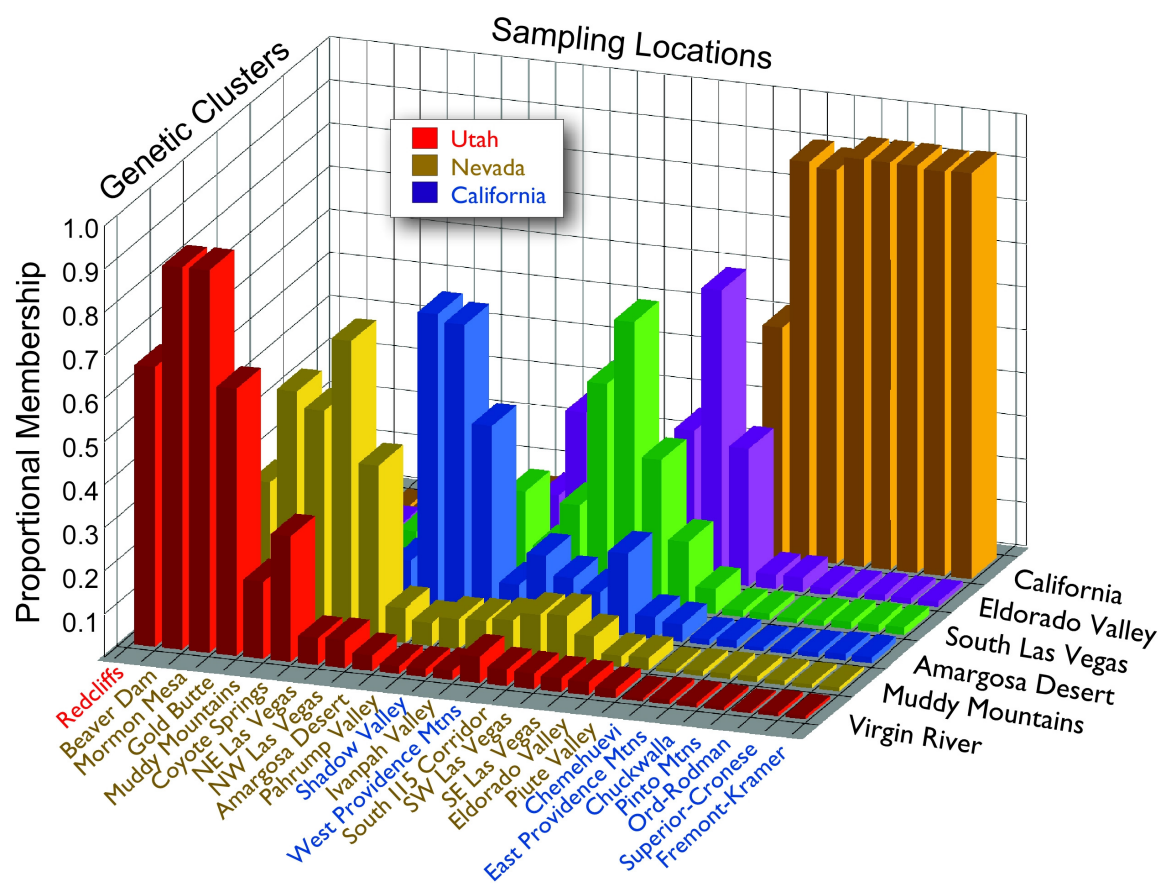


Fig 5a.

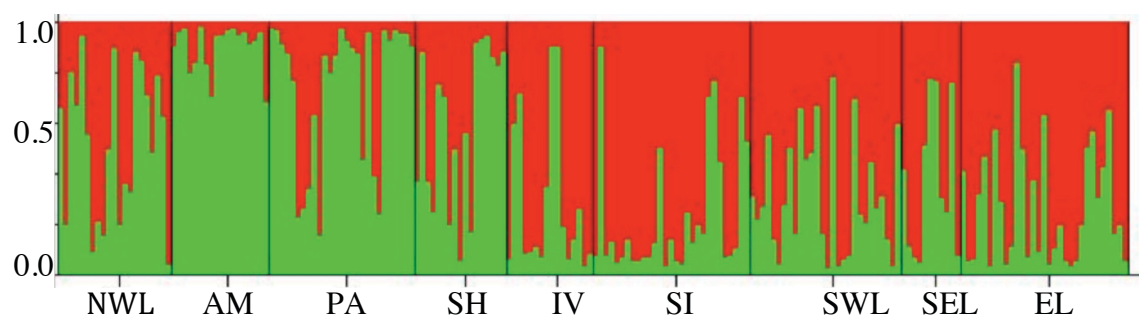


Fig 5b.

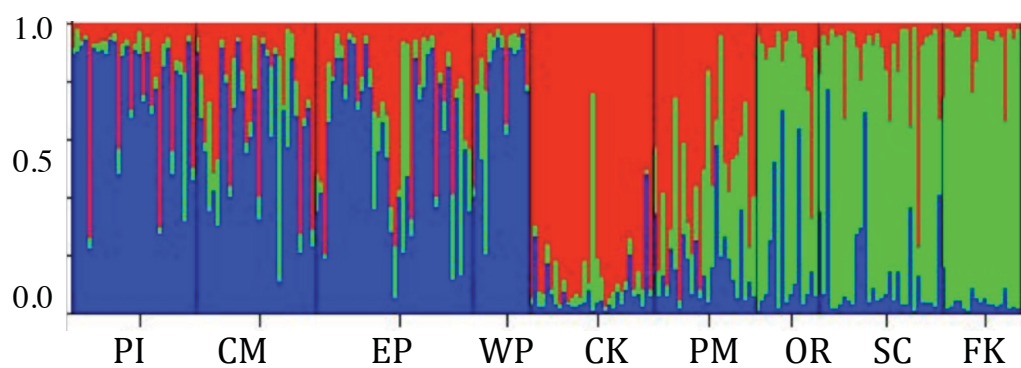


Figure 6.

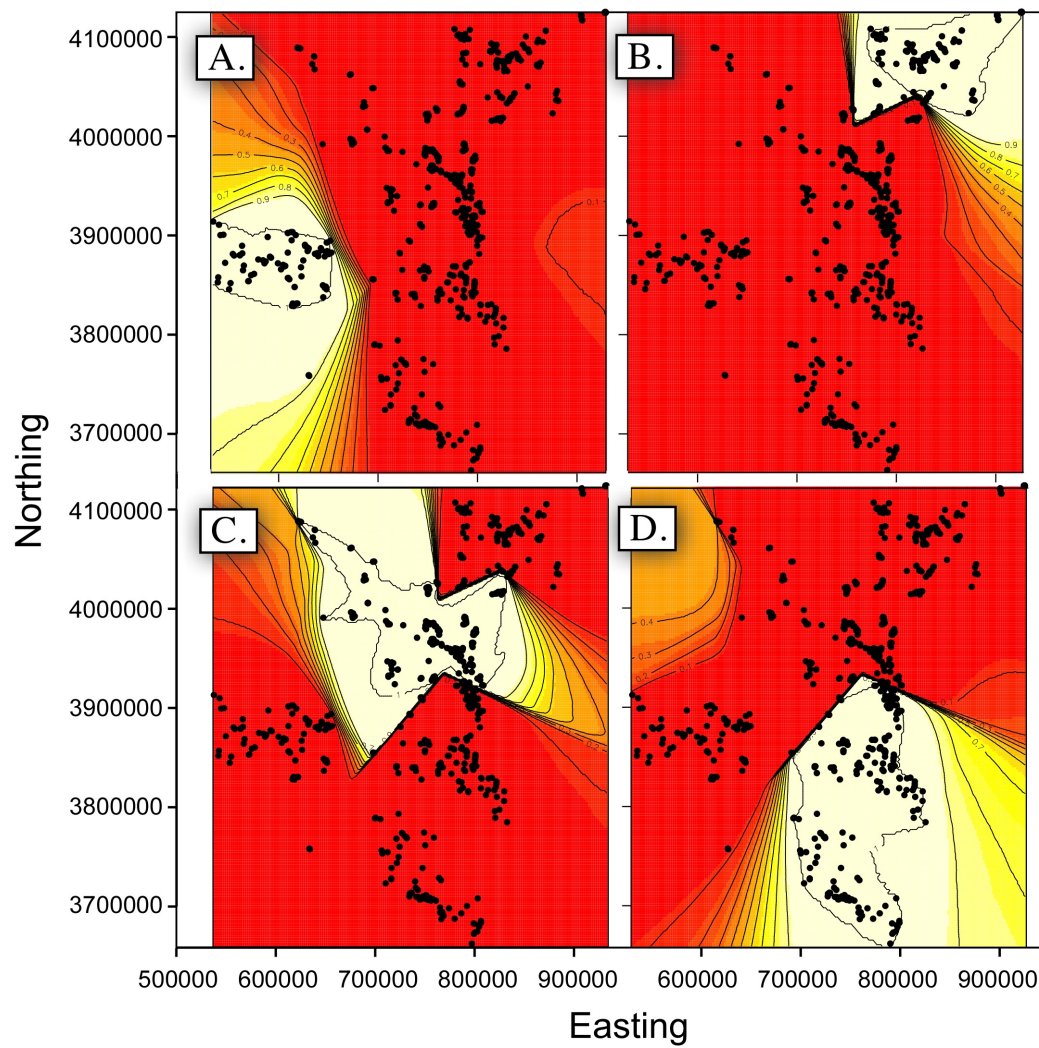
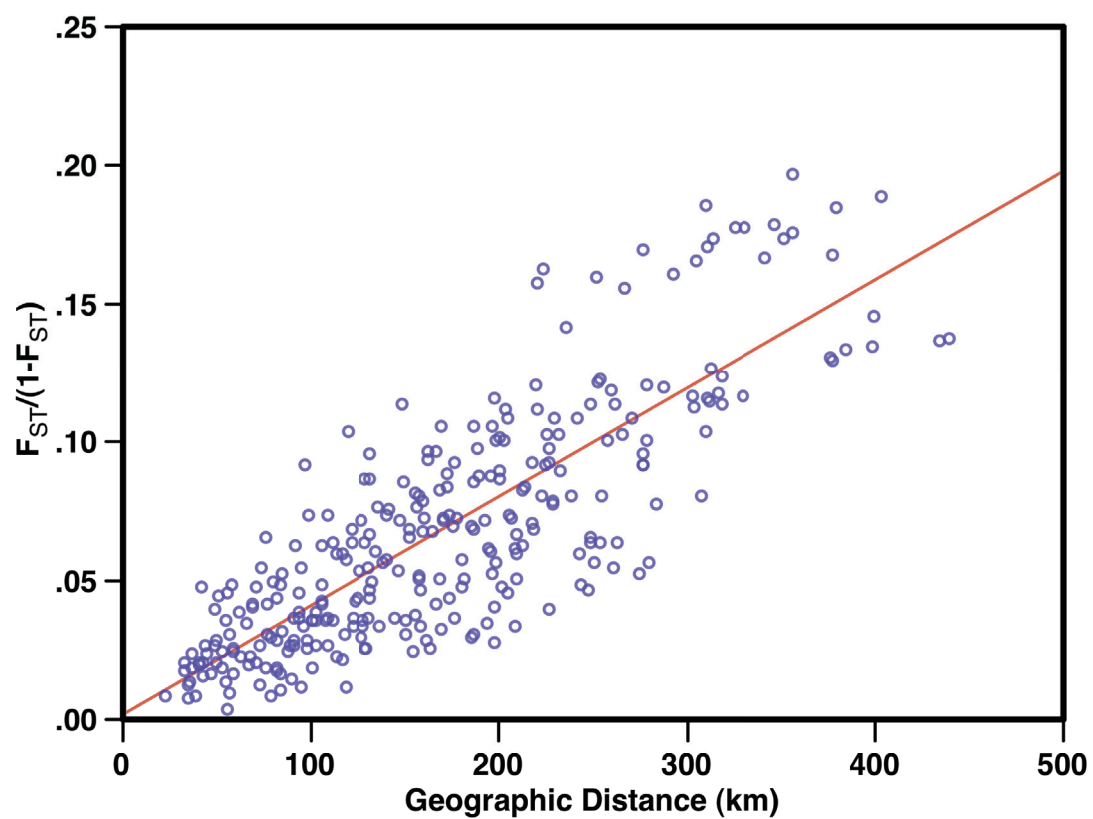


Figure 7.



**CHAPTER 2. MORE THAN ISOLATION BY DISTANCE: A LANDSCAPE  
GENETIC APPROACH TO IDENTIFYING THE POPULATION STRUCTURE  
OF THE MOJAVE DESERT TORTOISE**

**ABSTRACT**

Increasingly, land-use change and urbanization are fragmenting viable habitat for species across the planet, pushing landscape connectivity to the forefront of research in conservation biology. Heterogeneity in landscape features alters how an organism responds to and moves among habitat patches, affecting ecological and evolutionary processes such as dispersal and gene flow. Therefore, a detailed understanding of landscape connectivity is critically important to determine how population differentiation arises. In the absence of barriers to gene flow, geographic distance should explain genetic differentiation among individuals or local subpopulations. Isolation by distance has been identified as a main hypothesis for genetic differences within the Mojave population of the desert tortoise (*Gopherus agassizii*). Here, we used a landscape genetics approach to evaluate deviation from the isolation-by-distance model. We quantified landscape connectivity and genetic similarity in the Mojave desert tortoise. We tested multiple hypotheses to determine which environmental characteristics best correlate with patterns of gene flow. We compared four statistical models of suitable habitat containing biological and physical variables that could influence movement of tortoises through the landscape to a null model of movement of individuals through the habitat via a straight-line. To determine areas of structural connectivity, we used the “least cost path” and

“isolation-by-resistance” models. These two models provide different perspectives on landscape connectivity for the desert tortoise; however, both supported the hypothesis that topography was more influential in shaping patterns of gene flow than geographic distance alone. Life history characteristics of the desert tortoise influence gene flow and other processes, resulting in time lags in the visible patterns of gene flow. Therefore, we could not detect the effects of habitat fragmentation. Nevertheless, major interstates and urbanization have severed a majority of the potential habitat corridors desert tortoises use to move in the landscape. Wildlife passages and translocations may be necessary management actions to restore the high levels of gene flow that historically have occurred among desert tortoise subpopulations.

## **INTRODUCTION**

Maintaining and restoring linkages among populations and habitats is a conservation priority because many important ecological processes, such as dispersal, require connectivity (Crooks and Sanjayan 2006). Landscape connectivity describes the degree to which the landscape facilitates or impedes an organism’s movement, and contains a structural and functional component (Taylor et al. 1993, Brooks 2003, Taylor et al. 2006). The structural component includes the landscape heterogeneity that influences the habitat available to the organism; the functional component describes the organism’s response to the available habitat (Brooks 2003, Taylor et al. 2006). Quantifying both components is necessary to provide complete comprehension of how

organisms move through the landscape and the effects of removing linkages within the landscape.

Habitat fragmentation increases isolation among populations, and it is a key causal agent in the increase of extinction risk for many species (Crooks and Sanjayan 2006, Fischer and Lindenmayer 2007). The main causes of increased extinction risk include increased environmental and demographic stochasticity, increased numbers of deterministic threats, and loss of genetic variation (Lande 1988, Davies et al. 2001, Saunders et al. 2001, Fahrig 2003, Henle et al. 2004, Reed 2004, Fischer and Lindenmayer 2007). Although landscape connectivity should not be equated with increased population persistence (Taylor et al. 2006), it provides several clearly important means of reducing extinction risk (Crooks and Sanjayan 2006). Among other benefits, connectivity in the landscape allows dispersal from the natal range, aids in rescue effects to prevent local extinctions, facilitates gene flow that prevents inbreeding, and fosters adequate responses to environmental change through the potential for long-term adaptation, the ability to adjust the natural distribution, and proper response to disturbances (Crooks and Sanjayan 2006).

Although the most effective way to measure landscape connectivity is unclear (Moilanen and Hanski 2001, Tischendorf and Fahrig 2001), dispersal (or some measure of movement) is one common metric to evaluate the factors that facilitate connectivity and the consequences of the amount of connectivity (Wiens 2001, Uezu et al. 2005). Dispersal is a critical element in population ecology because it influences the distribution and abundance of organisms (Clobert et al. 2001), as well as the persistence of populations and metapopulations (Levins 1970, Hanski 1999). Although the importance

of dispersal is well-recognized, it is still poorly understood for many species (Wiens 2001). Direct assessments of dispersal are often mired in logistical constraints (Koenig et al. 1996, Mossman and Waser 1999). However, inferences from genetic data have been recognized as a viable alternative to direct measurements of dispersal (Koenig et al. 1996, Waples 1998, Bohonak 1999, Brooks 2003). Dispersal and gene flow are correlated in many natural systems, though the two processes are not synonymous because not all dispersers survive and reproduce (Bohonak 1999). Genetic data integrate movements with population level effects such as permanent emigration and/or breeding (Brooks 2003, Cushman et al. 2006, Epps et al. 2007, Keyghobhaldi 2007). Therefore, measurements of gene flow can be used to quantify functional connectivity (Brooks 2003, Stevens et al. 2006, Holdregger and Wagner 2008).

Determining of the components of landscape structure that facilitate or impede movement is critical for population ecology, evolutionary ecology, and conservation biology (Berggren et al. 2002, Damschen et al. 2006, Kareiva 2006). The emerging field of landscape genetics capitalizes on advances in geographic information systems (GIS) to analyze the relative contribution of different habitat variables to the amount of gene flow occurring within and among populations (Manel et al. 2003, Holdregger and Wagner 2006, Storfer et al. 2007, Holdregger and Wagner 2008). Using spatially explicit models combined with genetic data permits the testing of specific hypotheses regarding natural levels of connectivity, the influence of particular landscape features on individual movement, and the effects of habitat fragmentation (Manel et al. 2003, Keyghobaldi 2007, Storfer et al. 2007). The questions addressed are species-specific, and they are

constrained to the temporal and spatial scale at which individuals of a species experience their surroundings (Wiens 2001, Brooks 2003, Holdregger and Wagner 2008)

The landscape genetic approach is most valuable for populations that have continuous distributions (Manel et al. 2003). In the absence of barriers to gene flow in a continuous population, dispersal processes of the individuals in the species solely govern the spatial genetic structure (Slatkin 1993, Epperson 2003). The amount of genetic exchange relies upon the geographic distance separating subpopulations. Thus, increases in geographic distance result in a concomitant increase in genetic differentiation (i.e. isolation-by-distance; Wright 1943). For continuous populations, this process can be considered a null model of how genetic differentiation arises (Epperson 2003, Holdregger and Wagner 2006). Correlations of pair-wise estimates of genetic distance to Euclidean distance can be used to detect isolation-by-distance (Rousset 1997, Rousset 2000). Further, these measures can be used to detect deviations from the null model. Natural populations often depart from strict differentiation explained by geographic distance, suggesting that additional features within the landscape contribute to the observed differentiation (Arnaud 2003, Coulon et al. 2004, Spear et al. 2005, Broquet et al. 2006, Cushman et al. 2006, Epps et al. 2007). Modifying a strict model of straight-line distance among habitat patches by including features representing the heterogeneity of the landscape that an organism experiences has the potential to improve our understanding of landscape connectivity (Adriaensen et al. 2003, Theobald 2006).

Here, we evaluated deviation from the isolation-by-distance model and quantified landscape connectivity in the Mojave population of the desert tortoise (*Gopherus agassizii*). The Mojave desert tortoise can be considered an historically continuous

population that occurs north and west of the Colorado River in the southwestern United States (Germano et al. 1994). This portion of the species range is listed as threatened under the U.S. Endangered Species Act (USFWS 1994), and tortoise habitat in this region has become fragmented by transportation corridors, utility infrastructure, and urban development over the past century (Tracy et al. 2004). Although few data exist on dispersal of desert tortoises (Morafka 1994), a recent assessment of spatial genetic structure suggested that historic movement among adjacent subpopulations was extensive (Hagerty and Tracy in prep). The long generation time of tortoises provides a unique opportunity to detect natural population structure, which has now undoubtedly been disrupted. Genetic differentiation among subpopulations is small, although spatial structure is present (Hagerty and Tracy in prep). Strong correlations between genetic distance and geographic distance provide evidence that dispersal distances are a primary factor in governing gene flow. In fact, geographic distance explains approximately 65% of the variation in genetic distance (Hagerty and Tracy in prep, Murphy et al. 2007).

Despite this unusually strong evidence supporting the isolation-by-distance model, there is an expectation that particular features of the landscape facilitate or impede movement of desert tortoises. Certain landscape features, such as mountain ranges, are certainly potentially influential in structuring the population (Hagerty and Tracy in prep). Moreover, the increasing fragmentation of the Mojave Desert prompts increased interest in identifying the key components of landscape connectivity for this threatened species. The main objective of this study was to identify landscape features that influence the patterns of movement of the desert tortoise in the Mojave Desert. We investigated the effects of landscape features using a two-tiered approach. We used several models of

desert tortoise habitat to predict regions of habitat connectivity. Then, we evaluated the regions of connectivity by comparing the geographic distances that were modified by landscape features with genetic distances to determine if there is evidence that these features have influenced gene flow over a long temporal scale.

To quantify structural connectivity, we compared two modeling techniques: “least cost path” analyses and isolation-by-resistance analyses. “Least cost path” modeling has become one of the most common ways to investigate how landscape heterogeneity influences genetic distances among populations and individuals (Adriaensen et al. 2003, Spear et al. 2005, Theobald 2006, McRae and Beier 2007). The least cost path is an altered straight-line geographic distance between a pair of populations or individuals, which accounts for geographic features that may influence movements between two locations (Adriaensen et al. 2003, Theobald 2006). The outcome of the analysis is one optimal path that minimizes the cost of movement between the two individuals or populations. The isolation-by-resistance model incorporates the potential for multiple pathways between populations using a graph theoretic measure, which is based in circuit theory (McRae 2006, McRae and Beier 2007, McRae et al. 2008). This connectivity model has been shown to improve the ability to evaluate the effects of landscape heterogeneity on genetic structuring of populations because the model accounts for multiple regions of connectivity and irregular range shapes (McRae and Beier 2007).

We tested multiple hypotheses to assess which environmental characteristics best correlate with patterns of gene flow. Our null model was a simple model of straight-line geographical distance. Four models of suitable habitat for desert tortoises represent hypotheses alternative to straight-line movements, and these could bear on landscape

connectivity. These models identified the distribution of desert tortoise habitat using: (1) biological variables describing vegetation and precipitation, (2) physical variables describing elevation and slope, (3) combination of biological and physical variables, and (4) a binary habitat model. These a priori models were chosen to test specific hypotheses regarding the factors that are the most relevant in determining connectivity among tortoise habitat. First, we hypothesized that the modified distance measures will improve the amount of genetic variability explained by the null model of straight-line geographic distance. Second, we expected that the pattern of genetic distance for desert tortoises should better correlate with distance measures that incorporate topographical variables. Gene flow among tortoise subpopulations is mostly likely a slow cumulative process that occurs over longer temporal scales, which may not be affected by short-term environmental influences (e.g., measures of drought, annual plant production). Finally, the connectivity models based in circuit theory should outperform the least cost path models due to their ability to incorporate multiple paths of connectivity into a single landscape resistance measure.

## **MATERIALS AND METHODS**

### **Desert Tortoise Genotyping**

The Mojave desert tortoise inhabits portions of the Mojave and Colorado Deserts, spanning four states in the southwestern United States (Utah, Arizona, Nevada, and California; Germano et al. 1994). The Mojave and Colorado deserts ( $> 130,000 \text{ km}^2$ ) are

heterogeneous in climate, geology, and topography (Rowlands et al. 1982, Berry et al. 2006); however, the desert tortoise population is relatively continuous in the low-elevation (300 m – 900 m) regions dominated by creosote scrub (*Larrea tridentata*) vegetation (Luckenbach 1982). In the Mojave and Colorado Deserts, desert tortoises most commonly occur in areas with gentle slopes, sufficient shade resources, and friable soils to allow burrow construction (Bury et al. 1994, USFWS 1994 Andersen et al. 2000).

Between 2004-2006, whole blood was collected from 744 desert tortoises throughout the range where the species is federally listed, which includes areas north and west of the Colorado River (Table 1). Sample collection sites included areas sampled during annual population monitoring (USFWS 2006) along randomly placed transects within critical habitat and systematically-placed transects outside of critical habitat areas (Hagerty and Tracy in prep). Individuals were separated into 25 subjective sampling locations that were identified based on geographic location (Table 1).

Our laboratory procedures followed those described in Hagerty and Tracy (in prep). The 20 microsatellites used in this study were composed of loci originally developed for *Gopherus polyphemus* (Schwartz et al. 2003) and the Sonoran population of *Gopherus agassizii* (Edwards et al. 2003), as well as loci developed specifically for the Mojave desert tortoise (Hagerty et al. 2008). We amplified microsatellites and completed fragment analysis in collaboration with the Nevada Genomics Center (<http://www.ag.unr.edu/Genomics/>). All alleles were scored with GeneMapper 5.0 (Applied Biosystems, Inc.).

Deviations from Hardy-Weinberg equilibrium and linkage disequilibrium, levels of genetic diversity and population differentiation, and a complete description of

population genetic structure are described elsewhere (Hagerty and Tracy in prep). We calculated three pair-wise genetic distance measures for the 25 sampling locations:  $F_{ST}$  /  $(1 - F_{ST})$  (as recommended by Rousset 1997) using pair-wise  $F_{ST}$  values from FSTAT (Goudet 1996), the genotype likelihood ratio ( $D_{LR}$ ; Paetkau et al. 1997) in DOH (Paetkau et al. 1997), and Nei's standard genetic distance  $D_S$  (Nei 1972) in Tools for Population Genetic Analysis (TFPGA; Miller 1997). Additionally, we calculated  $D_{PS}$  (Bowcock et al. 1994) in MICROSAT (Minch et al. 1995) and  $a_r$  (Rousset 2000) using SPAGeDi (ver 1.2; Hardy and Vekemans 2002) as pair-wise measures of genetic distance for individual tortoises.

#### Straight-line Geographic Distance

We calculated pair-wise Euclidean distances (m) as a measure of straight-line geographic distance between pairs of sampling locations and individuals in ArcGIS (ver. 9.2, ESRI, Redlands, USA). Centroids of sampling locations were determined by calculating the central point in polygons defined for the 25 subjectively defined sampling regions in ArcGIS (ver. 9.2, ESRI, Redlands, USA). Universal Transverse Mercator (UTM) coordinates of individual locations were recorded when DNA samples were collected.

## Habitat Models for the Mojave Desert Tortoise

Typically, landscape resistance for connectivity modeling is determined using expert opinion or ad hoc measures using environmental variables (Adriaensen et al. 2003, Verbeylan et al. 2003, Broquet et al. 2006, Theobald 2006, McRae and Beier 2007). We chose to identify levels of landscape resistance with a model of the distribution of habitat in space as a replacement for expert opinion. The implicit assumption is that a model of habitat quality is a valid approximation for landscape permeability to dispersal (Broquet et al. 2006, Epps et al. 2007). Habitat models were developed previously for the Mojave desert tortoise using presence data collected throughout the Mojave desert in California and parts of Arizona, Nevada, and Utah (Thomas et al. in review). To create a spatial distribution of predicted habitat for desert tortoises, we chose the Generalized Regression Analysis and Spatial Prediction (GRASP) modeling algorithm, which uses regression (generalized additive models) to establish relationships between species occurrence and environmental variables (Lehmann et al. 2002). This model also requires pseudo-absence data points, which were formulated using a random selection of points from an area constrained using Ecological Niche Factor Analysis in Biomapper (Hirzel et al. 2002a, Hirzel et al. 2002b).

We chose six environmental variables for the habitat models, which we reduced from 16 spatial datasets consisting of biological and physical features identified as potential descriptors of desert tortoise habitat using classification and regression tree analyses (Thomas et al. in review). Model accuracy for the 16-variable and 6-variable GRASP models was extremely similar in most assessments (Thomas et al. in review).

Therefore, we chose the 6-variable model because it contained the most parsimonious set of explanatory variables. The six variables were: average surface roughness (a measure of topographic relief; Jenness 2002, Thomas et al. in review), elevation, annual plant cover (Wallace et al. 2006, Wallace and Thomas in review), mean dry (summer) season precipitation for 30-yr normal period (1961-1991), mean wet (winter) season precipitation for 30-yr normal period (1961-1991), and the spatially distributed coefficient of variation for wet season precipitation (1913-2004; Blainey et al. 2007) (see Thomas et al. in review for a complete description of environmental variables).

To assess the relative contribution of the physical and biological variables to explaining gene flow patterns, we created four different habitat models using specific combinations of the six variables listed above: (1) biological and physical model (six variables as in Thomas et. al in review), (2) biological only model (four variables), (3) physical only model (two variables), and (4) binary model (reclassified six variable model) (Fig. 2). To create the binary model, we reclassified the six variable model using the “precision recall break even” statistic as a threshold (Sing et al. 2005). The precision recall break even point is a commonly used method similar to the receiver operator curve, and it is defined as a trade off between precision (the number of presence points identified correctly) and recall (the total number of points) (Fawcett 2004, Sing et al. 2005). As a result, all grid cells contained a binary indicator of habitat (1 = habitat, 0 = not habitat). To calculate the least cost path for the binary model, non-habitat was coded as “no data” in the raster file, which caused those cells to be complete barriers to movement.

Each grid cell in the other three models contained a floating-point value that corresponded to the probability of desert tortoise occurring in that cell as predicted by the GRASP model. The inverse prediction of each of the habitat models was used as a measure of resistance to movement in the landscape, which was used to create a resistance layer in each connectivity analysis (least cost path and isolation-by-resistance). We analyzed each model type with two location parameters: (1) centroids of tortoise sampling locations ( $n = 25$ ), and (2) individual locations of desert tortoises ( $n=700$ ).

The area covered by the GRASP model included most of the range of the Mojave desert tortoise, except for a region in the Eastern Colorado Desert (southern tip of Chuckwalla Bench), the area in the extreme west of tortoise distribution near Edwards Air Force Base, CA, and the northern tip of the range in Southern Utah (Thomas et al. in review). We removed individuals from the data set when their locations were outside the boundary of the habitat models. As a result of the reduced area of the habitat model compared to the area sampled for tortoises, 44 desert tortoises were removed from the analyses ( $n = 700$ ). Four additional individuals were removed from the least cost path models because they were located on the edge of the cost surface and the least cost path could not be calculated (final  $n = 696$  for least cost path model).

## Two Models of Landscape Connectivity

### Least Cost Path

“Least cost path” analyses are used to determine the most likely path an individual should travel and they also can be used to estimate the effective distance

between habitat patches (Adriaensen et al. 2003, Theobald 2006). The effective distance is a modified Euclidean distance that uses landscape resistance to determine a more ecologically-relevant, optimal path between patches (Verbeylan et al. 2003, Theobald 2006). Typically, effective distance is calculated using a cost-weighted function (cost associated with moving across a cell). This simple algorithm sums the cost of moving from the beginning point to the end point. Movement can occur in the cardinal directions as well as along the diagonals (eight-neighbor algorithm). For diagonal directions, the cost is multiplied by a factor that varies based on the size of the cell to compensate for the longer distance. The cells in the habitat suitability raster maps were 1 km x 1 km. A graph theoretic structure is used for “least cost path” models when the landscape is represented as a raster map (Urban and Keitt 2001).

“Least cost path” models were calculated in GRASS (ver. 6.3). We used Program R (ver 2.7.1; R Development Core Team, 2008) to create instructions for running the models in GRASS. We also used Program R to manipulate the output from GRASS, rearranging the data into a matrix format. Two input files, a source layer containing UTM coordinates (population centroids or individual locations) and a resistance layer, were necessary to calculate the effective distance matrix and least cost path. The least cost path for each pair of tortoise location was quantified in two ways: (1) the cumulative cost across all cells while moving from location A to B (effective distance), and (2) the physical distance (i.e., number of 1 km<sup>2</sup> grid cells) traversed along the least cost path between location A and B (length of least cost path). We created a pair-wise matrix of the cost values and distance values to compare with the genetic and geographic distance

matrices using Mantel tests (Mantel 1967, Smouse et al. 1986). Finally, we mapped the least cost path between each of the 25 sampling locations in ArcGIS (ver. 9.2).

### Isolation-by-Resistance

“Isolation-by-resistance” connectivity models are based in circuit theory and they use a graph theoretic approach to predict movement patterns and quantify the effects of certain landscape features (McRae 2006, McRae et al. 2008). Graph theory allows connection throughout a network between a series of nodes (connection points that here can be equated to the centroid of a sampling location or an individual tortoise; see Urban and Keitt 2001 for a review). Connections between each node are edges, which can be weighted based on the strength of the connection (the number of dispersers exchanged; McRae et al. 2008). For isolation-by-resistance models, the edges in a graph network are represented as resistors in an electrical circuit. The amount of current ( $I$ ) that flows between resistors depends on the voltage ( $V$ ) applied and the resistance ( $R$ ) using Ohm’s law,  $I = V/R$ . The configuration and the resistances of each resistor also change the amount of current that flows. The resistance (reciprocal of conductance) can be thought of as the isolation or movement cost between nodes (McRae et al. 2008).

The metric used to measure connectivity in the landscape is the resistance distance. This value is the probability of moving from one point (population centroid or individual location) to another as the conductance value (measure of habitat quality) for a cell divided by the sum of the conductance values of all the cells connected to the point. The calculation of resistance distance between all desert tortoise locations was implemented in the Matlab Beta version of Circuitscape (provided by B. McRae). The

probability of occurrence in each grid cell was treated as a conductance value (the inverse is resistance). Movement can occur in the cardinal directions as well as along the diagonals (eight-neighbor algorithm). Resistance between a pair of first order neighbors (cardinal directions) was set to the average of the two cell's resistance values. The resistance between a pair of second order neighbors (along diagonals) was set to the average resistance multiplied by the square root of 2 to reflect a greater distance between cell centers (McRae et al. 2008).

Within the Circuitscape model, locations are dropped if they are not connected to other locations via grid cells. Grid cells with a conductance value of zero prevented connection between neighboring points. Therefore, we replaced all zero conductance values with 0.000001 in all habitat suitability models, except for the binary threshold model. Replacing the grid cells with zero values did not change the resulting resistance matrices, except to prevent the removal of locations to allow comparison with the “least cost path” models. We determined the number of nodes pruned between the input models with and without zeros for comparison. Program Circuitscape provided a pair-wise resistance distance matrix for all points of interest as well as a cumulative current map that can be viewed in ArcGIS ver. 9.2 (ESRI, Redlands, CA). The current map can be interpreted as predicting landscape corridors, which have an increased probability of dispersers using those areas.

## Model Comparison

We used Mantel tests (Mantel 1967) and partial Mantel tests (Smouse et al. 1986) to correlate genetic distance with a Euclidian distance, cost distance (cumulative cost or length of the least cost path), or resistance distance matrix. We completed Mantel and partial Mantel tests in Program R using the vegan package (Legendre and Legendre 1998). A Pearson product moment correlation was calculated, and significance was determined, by 10,000 permutations of the first matrix (Euclidean, resistance, or effective distance), holding the second matrix (genetic distance) constant. The model with most support will have highest simple correlation with genetic distance, and a significant positive correlation with genetic distance after controlling for Euclidean distance. Although partial Mantel tests have been criticized recently for potentially underestimating Type I error rates (Roufase and Rousset 2001, Rousset 2002), these criticisms could be overstated (Castellano and Balleto 2002). Additionally, we used the same second predictor variable (Euclidean distance) in all tests and we did not compare p-values, reducing the chance of bias in our interpretations (Epps et al. 2007).

## RESULTS

Relationship between Landscape Heterogeneity and Genetic Distance: Isolation-by-distance

Populations:

Euclidean distance correlated significantly with pair-wise genetic distances, as evidenced by a simple Mantel correlation (Table 2). Correlations between Euclidean distance and different measures of genetic distance were very high and ranged from 0.816 to 0.826 (Table 2), with the relationship being the strongest for  $F_{ST}/(1-F_{ST})$ .

Individuals:

Euclidean distance among all pairs of individuals was significantly correlated to genetic distance (Table 3); however, the correlation ( $r = 0.323$  and  $0.347$ ) was lower among pairs of individuals than between pairs of population centroids (Table 3).

Relationship between Landscape Heterogeneity and Genetic Distance: Least Cost Path

Populations:

Modified distances for each of the four habitat models were also correlated significantly with genetic distances between pairs of populations (Table 2). The distance traversed along the least cost path (length of least cost path) was better correlated to genetic distance than the effective distances (cumulative cost of the least cost path) in every case (Table 2). The length of the least cost path also was more highly correlated

with Euclidean distances between population centroids ( $r = 0.906 - 0.976$ ) compared to the effective distances ( $r = 0.659 - 0.947$ ). The length of the least cost path for the biological model had the second highest correlation, and was only surpassed by the distance traversed in the physical habitat model. The binary model had the lowest correlation with genetic distance (Table 2).

When Euclidean distance was held constant using partial Mantel tests, a majority of the effective distances and lengths from each of the habitat models were no longer significantly correlated with genetic distance (Table 4). The length of the least cost path in the physical model was significantly correlated with genetic distance using the  $D_{LR}$  measure and marginally significant using  $D_S$ , however the relationship was not significant for  $F_{ST} / (1 - F_{ST})$ .

The cumulative least cost paths across the 25 population centroids were similar for the four habitat models (Fig. 3). For example, all paths avoided large areas of unsuitable habitat such as the northwest corner of the range and New York and Providence Mountains. However, each set of least cost paths contained slight differences that correspond to differences in the underlying habitat suitability models (Fig. 3). For example, individual paths for the biological model crossed portions of the Spring Mountains, Death Valley, and the Baker Sink (Fig. 3). The combined model and binary model did not have a direct path between Amargosa Desert and Northwest Las Vegas Valley, which was present in the individual biological and physical models (Fig. 3). The least cost paths in the binary model covered more area in regions like the Western Mojave, but had more restricted paths in Las Vegas Valley and in the Northern Mojave near the Amargosa Desert (Fig. 3).

The large regions of unsuitable habitat in the binary model restricted where paths could exist, and caused five populations to be removed from both connectivity analyses (Fig. 3 and Fig. 4). The northern portion of the range (including Red Cliffs Desert Reserve and Mormon Mesa) was almost completely isolated from the remainder of the range according to this model. Additionally, Coyote Springs, Muddy Mountains, and the West Providence Mountains were removed. The placement of the centroids undoubtedly affected if points were completely removed from the least cost path model (Fig. 3). The same populations were also removed in the isolation-by-resistance models (Fig. 4).

#### Individuals:

The pattern of correlations between the length and cumulative cost of the least cost path and genetic distance was similar to the patterns with population comparisons. As with Euclidean distance, the correlations were weaker, but significant (Table 3). Distance-traversed in the physical model had the highest correlation ( $r = 0.348$ ), followed by the biological model, and combined model (Table 3). The least cost path for the binary model had the lowest correlations (Table 3). In a majority of tests,  $a_r$  had higher correlations than the  $D_{ps}$  genetic distance measure (Table 3). After accounting for Euclidean distance, distance-traversed in the full model, physical model, and binary model, as well as the cumulative cost of the biological model and binary model, were significantly correlated with genetic distance (Table 5). Additionally, the biological cost model had the highest partial correlation with genetic distance (Table 5).

## Relationship between Landscape Heterogeneity and Genetic Distance: Isolation-by-Resistance

### Populations:

Resistance distances from three of the four habitat models were significantly correlated with genetic distance; however, the simple Mantel correlations were much lower than for the least cost path models (Table 2). Resistance distances calculated from the combined biological and physical habitat model were not correlated significantly to genetic distance (Table 2). After accounting for variance explained from Euclidean distance in the partial Mantel test, three of the four resistance distance matrices no longer correlated significantly with genetic distance (Table 4). However, the threshold model had the highest partial Mantel correlations among all tests and remained significantly correlated with two of the three measures of genetic distance ( $D_{LR}$ ,  $D_S$ ).

The cumulative current maps for the biological, physical, and combined habitat models showed similar areas of high density current (Fig. 4). However, the currents were more diffuse in the biological model (Fig. 4). For example, more low current connections were apparent between the Amargosa Desert and northwest Las Vegas Valley. In comparison, only one strong connection was clear in the physical model. Certain physical barriers did not show any current flow in any habitat model, including the Spring Mountains and the New York and Providence Mountains (Fig. 4). Additionally, the northern portion of the desert tortoise's range in Nevada and into California, mainly through Las Vegas valley contained areas of very high current density (Fig. 4). Natural

barriers did not fragment habitat within California and had more diffuse current flow between sampling locations (Fig. 4).

As described in the results for the least cost path models, the binary habitat model contained large patches of unsuitable habitat, which caused several population points to be completely isolated from other sampling locations in the isolation-by-resistance model (Fig. 4). Certain barriers were more apparent in the current map using the binary habitat model, which were not as visible in the other three models (Fig. 4). For example, the Baker sink is visible as an area with no current flow in this model. Other barriers (such as the Spring Mountains) that were visible in the other three models are more exaggerated in the binary model (Fig. 4). Regions such as Las Vegas Valley also have high current density in this model.

#### Individuals:

The pattern of correlations between resistance distance and genetic distance for individuals also was similar to the patterns with population comparisons. The physical model had the highest correlation, followed by the threshold, biological, and combined model (Table 3). Many of these correlations were not significant after accounting for Euclidean distance; however, the threshold model was significant and had the highest partial Mantel correlation (Table 5). The biological and combined models were correlated significantly to Rousset's  $a_r$ .

## DISCUSSION

The goals of this research were to determine to what extent landscape features improve understanding of variation of genetic distances among desert tortoise populations, and to identify potentially important habitat corridors among tortoise habitat in the Mojave Desert. We treated isolation-by-distance as our null hypothesis, which would be the dominant process in the absence of barriers in a continuous population (Epperson 2003). We tested multiple models, which altered the straight-line distances by accounting for the cost of movement through the landscape based upon biological and physical variables. Previously these variables were used to predict successfully desert tortoise occurrence in the Mojave Desert (Thomas et al. in review). Additionally, we forced the full model to be binary habitat (occupancy and no occupancy), which greatly reduced the available habitat that could be used as a corridor.

Measuring landscape connectivity is an active area of research and there is no consensus on the most effective way to identify the variables contributing to observed (or inferred) movement patterns (Tischendorf and Fahrig 2000a, Tischendorf and Fahrig 2000b, Moilanen and Hanski 2001, Tischendorf and Fahrig 2001). Therefore, we compared the least cost path model to the isolation-by-resistance model, both of which have been used successfully in other systems (Coulon et al. 2004, Broquet et al. 2006, Cushman et al. 2006, McCrae and Beier 2007). Through this approach, we were able to identify more rigorously the landscape features that best correlate with genetic distance. Our models provided evidence that habitat variables indeed influence gene flow and hence historical dispersal of desert tortoises.

## Model Comparisons

Straight-line geographic distance between sampling locations of desert tortoises strongly correlate with genetic distances, suggesting that dispersal distance is a major factor shaping genetic structure among and within populations (Edwards et al. 2004, Murphy et al. 2007, Hagerty and Tracy in prep). Further, desert tortoises have been deemed a model organism for studying this phenomenon (Edwards et al. 2004). However, heterogeneity of desert tortoise habitat, and the genetic structure of the subpopulations, suggests that other factors also may influence dispersal, and hence gene flow. Our data do support the null model of isolation-by-distance as an explanation for the observed patterns of genetic differentiation in desert tortoises. This circumstance is unusual. A majority of landscape genetic studies for terrestrial species have determined that straight-line distances are correlated only weakly with genetic distance (Vos et al. 2001, Arnaud 2003, Coulon et al. 2004, Spear et al. 2005, Broquet et al. 2006, McCrae and Beier 2007).

Although straight-line geographic distance was correlated strongly with our indirect measures of gene flow, the added movement costs accounting for landscape heterogeneity in both connectivity models improved the correlation. Generally, all connectivity models for all habitat variables for populations and individuals were significantly correlated with genetic distance. These correlations are not surprising because all the variables were correlated with geographic distance. Overall, the length of the least cost path (through a cost surface produced with elevation and average surface

roughness) had the highest correlation with genetic distance for populations and individuals. When Euclidean distance was parsed out, the correlations remained significant. These significant and strong correlations provide general support for our hypothesis that the temporal scale at which gene flow occurs in desert tortoise populations causes topographical variables such as elevation to be more influential.

The modeling approach that we chose affected the strength of the correlations for each type of landscape variable; therefore, the model(s) with the most support varied between the “least cost path” and “isolation by resistance” models. After accounting for straight-line distance using the partial Mantel tests, different variables were best correlated with genetic distance depending upon the connectivity model used. When the length of the least cost path among populations was calculated, physical habitat characteristics were more valuable. Additional least cost path models had support among the individual analyses, including the effective distance of the biological model and the binary models. When a circuit-theory model was used, the binary model explained more variation in genetic distance among populations and individuals. Barriers related to topographic relief, such as the Spring Mountains, New York Mountains, Providence Mountains, and Baker Sink, were more visible when binary habitat was used to model connectivity (Fig. 3 and Fig. 4). Additionally, the partial correlations for this model was stronger than any other partial correlation using either approach.

Binary descriptors of habitat may be better suited for the isolation-by resistance modeling. In some instances, current maps from habitat models that contained a floating-point value between 0 and 1 as a measure of conductance or resistance in each grid cell showed diffuse connectivity across the majority of the surface (not shown). The current

map for the binary habitat model was a stark contrast to the other habitat variables, clearly showing multiple distinct paths around potential barriers (Fig. 4).

Contrary to our expectations, the least cost path models consistently more strongly correlated with genetic distance than did the isolation by resistance models for pairs of populations. Previous comparisons of these two models provided evidence that the circuit theory models greatly improved the amount of genetic differentiation explained by landscape heterogeneity (McCrae and Beier 2007, McCrae et al. 2008). With empirical and simulated data, the circuit theory model can account for range shape (and irregularities in habitat extent), which could profoundly affect genetic structure (McCrae and Beier 2007). Mimicking movement in natural populations, the isolation by resistance model accounts for multiple pathways and habitat corridors differing in width between populations (McRae 2006, McRae et al. 2008). As a result, this modeling approach seems superior in landscape genetics and for conservation planning.

Despite the theoretical benefits, the resistance distance did not consistently improve our ability to measure functional connectivity quantitatively, suggesting that there may be limitations to the approach in its current state. However, the corridors detected by the isolation-by-resistance model using binary habitat did explain additional variation beyond Euclidean distance, and had the highest overall partial Mantel correlation. The reduced habitat available for connectivity in the binary model emphasizes geographic barriers and may have increased the importance of redundancy of habitat corridors in certain areas. This ability to include redundancy in habitat corridors in the circuit-theory models sets it apart from “least cost path” models (McRae et al. 2008). Therefore, this particular isolation-by-resistance model appeared to be more consistent

with patterns of gene flow. Identifying these potential corridors also provides key information for future conservation decision-making and hypothesis formation.

Least cost path models also provide valuable information on the landscape features that affect gene flow in desert tortoises. The physical habitat variables (elevation and average surface roughness) produced optimal paths through the landscape that closely resembled patterns of genetic distance among populations. Within this set of models, the length of least cost path was better correlated with genetic distance than was the effective distance. Although the shape of the cost surface appears to have approximated the pathways where gene flow occurs, the actual cost values reflected in the cumulative cost of each path were not as successful. The actual landscape resistance values from the habitat suitability model may reflect habitat use and not the cost of dispersal (Epps et al. 2007). The effective distances among individuals for the biological model were exceptions to this general observation. There are several potential explanations for this result. The large sample size in the individual analyses may have caused biologically unimportant models to be significantly correlated to genetic distance after accounting for Euclidean distance. Alternatively, the population models had small sample sizes that may have prevented identification of an important biologically relevant relationship. Beyond sample size, the genetic distances among individuals may indeed be related to biological variables such as precipitation and vegetation cover. Further, this relationship may be undetectable when summarizing genetic distance among individuals from a large geographic area.

## Spatial and Temporal Influences on Long-term Movements in Desert Tortoises

Both approaches to modeling connectivity supported the hypothesis that landscape variables, especially geographic barriers, modify desert tortoise movements, and explain additional variation beyond the null model of isolation-by-distance. Elevation and average surface roughness were identified as strong indicators of deviation from straight-line tortoise movement. These variables likely are also indirect measures of several factors that directly impact how individual tortoises traverse the landscape. Topography influences the thermal environment, soil type, and vegetation assemblages available for forage and shelter, which are a few of the many factors that appear to impact tortoise occurrence and activity (Nagy and Medica 1986, Bulova 1994, Germano et al. 1994, Zimmerman et al. 1994, Hilliard 1996, Duda et al. 1999, Anderson et al. 2000, Nussear 2004). Very high and low elevation areas most likely impose thermal constraints coupled with reduced availability of protective cover and friable soils for burrow construction (Anderson et al. 2000). Actual physical impairment to movement also likely plays a role in causing high elevation regions such as mountain ranges to be barriers. These apparent high and low elevation barriers are visible in the isolation-by-resistance model that used binary habitat designations (Fig. 4d). Although the biological variables (vegetation cover and precipitation) did not have a strong relationship with the pattern of gene flow among desert tortoise populations, these variables were at least equally important among individuals and proved to be effective in habitat suitability modeling. Additionally, these variables may be considered a short-term predictor of desert tortoise occurrence (Thomas et al. in review).

Temporal scale is important to consider when using genetic structure to seek meaningful factors contributing to landscape connectivity (Theobald 2006, Keyghobadi 2007). Genetic exchange and dispersal are population-level processes that occur over long temporal scales (decades to centuries, especially for species with long generation times). For longer temporal scales, the number of linkages or corridors decreases (i.e. there are more linkages for daily movements; Theobald 2006). Therefore, we would not expect the biological model to be highly correlated with patterns of gene flow because those variables would be more closely associated with movement over shorter time periods (e.g. 30 years). Our results support the hypothesis that topographical variables should predict areas of landscape connectivity that are more closely associated with genetic distances because elevation and slope remain relatively similar over geologic time, and gradually and consistently influence genetic structure. Additionally, any changes in gene flow may not be visible in genetic structure due to a considerable time lag that is influenced by effective population size and substructure (Wright 1943, Varvio et al. 1986, Waples 1998, Cushman et al. 2006). Further, the life history traits of the desert tortoise also affect the time lag that will cause inertia to changes in genetic population structure. The desert tortoise has a long life span, long generation time, and increased period of influence, due to the potential for reproduction during a very long adult lifespan. All of these characteristics are likely to obscure evidence of recent changes in gene flow (Keyghobaldi 2007). Therefore, patterns of gene flow that we observed were generally a cumulative signature of movements over many generations and not reflecting potential recent changes.

## Conservation Implications of Landscape Connectivity

The landscape connectivity models presented here indicate historical processes that occurred in the Mojave population of the desert tortoise. Therefore, we appropriately did not include roads and urban areas in the habitat models and connectivity models. These efforts provide a unique opportunity to identify patterns of connectivity that existed prior to recent anthropogenic changes to the Mojave Desert. Particularly for species with long generations times (such as the desert tortoise), detection of the effects of habitat fragmentation often are not possible, even with the use of variable molecular markers (Keyghobadi 2007). A recent study of population structure in the Mojave Desert tortoise noted that any changes in gene flow that has occurred over the past century were not visible even with microsatellite markers (Hagerty and Tracy in prep). Anthropogenic causes of habitat fragmentation, such as roads and land use changes, likely have caused imperceptible effects in genetic analyses because most roads were absent over the relevant temporal scale (Cushman et al. 2006, Keyghobadi 2007). However, some evidence exists that roads will cause changes in genetic structure with sufficient time (e.g., Gerlach and Musolf 2000, Vos et al. 2001, Epps et al. 2005).

Understanding historic ecological processes should shape management actions implemented to maintain or restore natural connections within the Mojave population of the desert tortoise. Connectivity may have been historically high through Las Vegas Valley and along the east and west side of the New York and Providence Mountains into California (Fig. 4). Las Vegas Valley was hypothesized to be a transitional corridor between habitat in the northern and southern reaches of the range (Britten et al. 1997, Hagerty and Tracy in prep). Although barriers are present, mainly in the form of major

mountain ranges, connections were most likely possible through local interactions over long time periods. Therefore, most, if not all, barriers were permeable over the long temporal scale at which tortoise population dynamics occur. Further, the population structure previously described from individual-based assignment tests indirectly supports the barriers and corridors identified in this study (Hagerty and Tracy in prep).

Desert tortoise habitat in California did not have equivalent heterogeneity in topographic relief, causing the habitat to be more continuous and showing few “pinch points.” The low-elevation region, known as the Baker Sink, is visible as a barrier separating the Northern and Eastern Colorado in the most conservative model (binary habitat), however, connected habitat in this model was reduced so severely that many regions that are known to have desert tortoise were removed. Areas, such as habitat in Southwestern Utah, were likely connected in the past, suggesting that the binary model underestimates historic areas of connectivity.

Although the effects of habitat fragmentation via roads and other virtually impassible human-caused barriers for tortoises would not be evident from our genetic analyses, we can generate hypotheses based on inferences about historic levels of connectivity and knowledge of current levels of fragmentation. The direct and indirect impacts of paved and unpaved roads and other effects of urbanization on desert tortoise populations are numerous and widespread (USFWS 1994, Boarman 2002, USFWS 2008). Roads indisputably cause direct mortality of individuals (Boarman et al. 1996, Boarman and Sazaki 2006), increase the potential for human contact with tortoises (Tracy et al. 2004), increase the spread of invasive plants (Lovich and Bainbridge 1999, Brooks and Berry 2006), and prevent individuals from moving among subpopulations (Boarman

et al. 1997, Edwards et al. 2004, Boarman and Sazaki 2006). Within the Mojave Desert, roads and other anthropogenic impacts have increased dramatically in recent decades (USFWS 1994, Lovich and Bainbridge 1999, Hunter et al. 2003, Tracy et al. 2004, USFWS 2008). Busy roads are a specific concern for landscape connectivity because they can be filters or complete barriers to movement (Clevenger and Wierzychowski 2006).

We can deduce from population genetic analyses that movement among subpopulations within the Mojave Desert was high. Desert tortoises exhibit low genetic differentiation among nine sub-populations within the Mojave Desert and Colorado Desert (Hagerty and Tracy in prep). Inferences from genetic data complement the understanding that tortoises have the capability to make long-distance forays for foraging and reproduction. Although dispersal ecology for this species is not well understood (Morafka 1994), anecdotal evidence suggests individuals can move long distances (> 30 km; Edwards et al. 2004). Large corridors are evident in the isolation-by-resistance models, providing additional support for the hypothesis that dispersal distances are as large or larger than anecdotes imply.

Currently, active management occurs within ten large expanses of habitat (Desert Wildlife Management Areas) across the listed range (USFWS 1994). A majority of these managed areas are isolated by, or bisected by, interstate freeways, and many main roads have been fenced to prevent tortoises from entering the highways where they will be killed. Although fencing has effectively reduced mortality (Boarman et al. 1996, Boarman et al. 1997), this management action has drawbacks in that it further fragments habitat and halts potential movement among previously connected subpopulations (Ruby

et al. 1994, von Seckendorff Hoff and Marlow 2002, Edwards et al. 2004). Coupling fencing of roads with culverts to allow natural movement under roads may be an effective combination to reduce mortality and maintain connectivity (Clevenger and Wierzechowski 2006). Culverts and other types of wildlife passages have been successful for increasing movement across dangerous highways (Clevenger et al. 2001, Clevenger and Wierzechowski 2006). Limited research suggests culverts may be a viable alternative for tortoises (Fusari 1985, Ruby et al. 1994, Boarman et al. 1996, Boarman et al. 1997); however, long-term research is necessary to evaluate the success of culverts for maintaining connectivity among subpopulations (Clevenger and Wierzechowski 2006). Translocating individual tortoises to maintain genetic connectivity is another potential management action; however, the directionality and amount of dispersal necessary to preserve these connections is not clear (Frankham 2006).

#### Limitations of the Methods

##### Habitat Models:

The measures of habitat suitability used to reflect landscape resistance successfully predict the occurrence of desert tortoises throughout the Mojave Desert (Thomas et al. in review). However, these statistical habitat models only imply the potential causal factors that shape a species distribution (Austin 2002, Lehmann et al. 2002, Kearney and Porter 2004). Predictive habitat modeling requires extensive presence and absence data to quantify the extent of suitable habitat, as well as the “true” environmental variables (Lehmann et al. 2002, Zaniwski et al. 2002). When reliable

absence data are not available, as is the case with desert tortoises, pseudo-absences can be generated to model suitable habitat (Zaniewski et al. 2002, Lutolf et al. 2006, Chefaoui and Lobe 2008). Using Ecological Niche Factor Analysis (ENFA) to create constrained pseudo-absence points may create false positive occurrence points because these methods tend to increase the amount of predicted suitable habitat (Zaniewski et al. 2002, Engler et al. 2004, Thomas et al. in review). Constraining where absence points can be placed using a model that over-predicts habitat could result in an artificially large measures of suitable habitat (Thomas et al. in review). Improper characterization of habitat could result in inaccurate shape and areas of predicted connectivity. Continued advances in statistical modeling to closely mimic ecological processes (Austin 2002, Lehmann et al. 2002), as well as the use of mechanistic models to predict habitat (Kearney and Porter 2004), are prospective improvements for predicting and understand the abundance and distribution of species.

Although we were able to use statistical models to identify pertinent landscape features, we were not able to use the probability of occurrence successfully to calculate cumulative cost values that reflected genetic patterns. Other researchers have altered the resistance values to reflect expert opinion and they have tried a range of resistance values because true values were unknown (Verbeylen et al 2003, Cushman et al. 2006). The relative cost of certain landscape features appears to be most important to increasing accuracy (Adriaensen et al. 2003, Verbeylen et al. 2003). This approach may be more beneficial when the probability of occurrence provided by suitable habitat models does not closely correspond to landscape resistance. Additional research is necessary to investigate the association between habitat use (and tortoise presence or absence) and

dispersal costs (Stevens et al. 2006). Continued efforts to provide realistic estimates of dispersal costs through habitat modeling will be valuable for future understanding of landscape connectivity (e.g., Pither and Taylor 1998, Wiens 2001, Stevens et al. 2006). Further, direct measurements of costs related to desert tortoise movement will be required to support the results from these modeling efforts.

#### Connectivity Models:

Although the “least cost path” approach and the “isolation-by-resistance” approach are valuable in assessing the functional connectivity in a heterogeneous landscape, they have limitations. Both approaches rely on relevant landscape variables that accurately reflect the cost of dispersal for the organism. Therefore, the effectiveness of the approach depends upon the success of the data used to model resistance (Holdregger and Wagner 2008).

The “least cost path” approach assumes that the organisms are omniscient, and they have a complete understanding of the landscape (knowing all possible routes) and then choose the most efficient path (Stevens et al. 2006, McRae and Beier 2007). This assumption may have been less relevant in our study because the least cost paths are strongly correlated with the linear paths. The least cost path value also is independent of the size of the potential corridor, which may influence an organism’s decision to take a suggested route (Adriaensen et al. 2003). If there is only one reasonable path between habitat patches or populations, the least cost path model may be sufficient to model movement. However, when multiple routes are equally probable, the least cost path models may not be as effective (McRae and Beier 2007).

Measures of resistance distance in isolation-by-resistance models complement the effective distance in the least cost path approach. This measure integrates all possible pathways into the distance calculations, whereas the least-cost distance is measured along one single optimal pathway. If there is only one pathway available to the organism, the least cost distance and the resistance distance should be equal. However, if additional pathways are present, the resistance distance provides a measure of redundancy (McRae et al. 2008). The main limitation of the isolation by resistance model is that the circuit model does not appear to accommodate gradients of conductance or resistance to calculate resistance distances, at least in some circumstances. Additionally, the magnitude of resistance is the same in both directions and therefore, cannot accommodate asymmetric movement, which could be very important in source-sink populations (McRae et al. 2008).

#### Genetic Markers to Indicate Dispersal:

Genetic models rely on simplifying assumptions (e.g. drift-migration-mutation equilibrium), and deviation from equilibrium may prevent easy interpretation of effects of landscape features on genetic structure (Rousset 2001, Broquet et al. 2006,). Additionally, estimates of gene flow from highly variable genetic markers do not provide an exact measurement of dispersal (Rousset 2001, Coulon et al. 2004, Epps et al. 2007). Genetic markers provide a cumulative signature of movements followed by successful reproduction among subpopulations (or populations) over many generations (Brooks 2003, Keyghobaldi 2007). Therefore, inferences from our modeling are not mechanistic explanations for movement of individuals. Our models are best used for addressing large

scale patterns of gene flow that were present for generations, not the nuances of dispersal over short time scales (Epps et al. 2007). Additional information is required to link patterns of gene flow to individual behavior, and these studies can also be used to determine landscape resistance empirically (as described above).

We used individual and population measures of genetic distance to evaluate relationships between environmental factors and gene flow. Among population measures of genetic distance, Rousset's genetic distance ( $F_{ST}/(1-F_{ST})$ ; Rousset 1997) had a higher correlation with straight-line geographic distance, and did not have any significant correlation with landscape resistance measures despite having similar correlation values. The reason for the difference between this measure, and the other genetic distance measures is not clear. Individual genetic distances were variable and had much lower correlations with straight-line geographic distances as well as landscape resistances from both connectivity models. This lower correlation is consistent with other studies in landscape genetics that used genetic distances among individuals (Coulon et al. 2004, Broquet et al. 2006). Although population measures did not provide definitive results in previous studies in other systems (Cushman et al. 2006), we obtained interpretable results at both levels of analysis. Populations were also used successfully for vertebrates tied to landscape features in another system (Walker et al. 2007). Assuming homogeneity across large areas can cause distances between groups to be obscured, however, we chose 25 sampling locations, which can be divided into nine subpopulations (Hagerty and Tracy in prep). Using individual comparison represents the complexities of genetic structure and shows the deviations from the average comparisons among locations. Therefore,

individual comparisons may be more appropriate for systems with a strong isolation-by-distance component such as desert tortoises.

## CONCLUSIONS

Although straight-line geographic distance is correlated strongly with genetic distance, which corroborates previous studies, the landscape genetics approach provided additional insight on the ecology of desert tortoise movement. Modifying straight-line distance using topographical features of the Mojave Desert significantly increased the correlation between genetic distance and geographic distance. This improvement suggests that high-elevation mountain ranges and formidably hot and dry low-elevation areas influence dispersal of desert tortoises over long temporal scales. The two connectivity models provided different perspectives on landscape connectivity for the desert tortoise; however, both supported the hypothesis that topography was influential in shaping patterns of gene flow. Our inferences regarding connectivity should be viewed as historical, and they do not reflect current habitat connections in the Mojave Desert. We were not able to detect the effects of habitat fragmentation because genetic signatures of movements have huge inertia, but major interstates and other roads have severed a majority of inferred habitat corridors for desert tortoises. Wildlife passages and translocations may be necessary management actions to restore the high levels of gene flow that historically occurred among desert tortoise subpopulations.

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## TABLES

Table 1. Sampling locations based on geography (including the state and abbreviation for the site), and the number of individuals sampled from each location. Locations centroids were used to identify cost distance matrices and resistance distance matrices, as well as genetic distance matrices. (recreated from Hagerty and Tracy in prep)

Sampling location	Abr.	State	Number of samples
Red Cliffs Desert Reserve	RC	UT	33
Beaver Dam Slope	BD	UT, NV	12
Mormon Mesa	MM	NV	43
Gold Butte-Pakoon Basin	GB	NV, AZ	17
Coyote Springs	CS	NV	26
Muddy Mountains	MD	NV	30
Northeast Las Vegas Valley	NEL	NV	20
Northwest Las Vegas Valley	NWL	NV	21
Pahrump Valley	PA	NV	27
Amargosa Desert, Oasis Valley, Greenwater Valley	AM	NV, CA	18
Southwest Las Vegas Valley	SWL	NV	28
South I-15 Corridor (Goodsprings, Jean Dry Lake, Sloan)	SI	NV	29
Southeast Las Vegas Valley (River Mountains)	SEL	NV	12
Eldorado Valley	EL	NV	49
Piute Valley	PI	NV	80
Ivanpah Valley	IV	CA	16
Shadow Valley	SV	CA	17
East Providence Mountains	EP	CA	38
West Providence Mountains	WP	CA	14
Chemehuevi DWMA	CM	CA	59
Chuckwalla DWMA	CK	CA	56
Pinto Mountains	PM	CA	
DWMA/Joshua Tree NP			25
Ord-Rodman DWMA	OR	CA	14
Superior-Cronese DWMA	SC	CA	45
Fremont-Kramer DWMA	FK	CA	19

Table 2. Mantel correlations between pair-wise population Euclidean distance, resistance distance (IBR), or least-cost distance (cost or length) and genetic distance ( $F_{ST}/(1-F_{ST})$ ,  $D_S$ ,  $D_{LR}$ ). The Mantel test statistic  $r$  is based on Pearson's product-moment correlation and significance values are based on 10000 permutations. The binary matrices contained 20 populations (other points were completely isolated and dropped from the model).

\* significance at the  $p < 0.05$  level, \*\* significance at the  $p < 0.001$  level

Spatial distance	Genetic distance		
	$F_{ST}/(1-F_{ST})$	$D_S$	$D_{LR}$
Euclidean distance	0.826 **	0.816**	0.821 **
LCP – Biological (cost)	0.661 **	0.673**	0.629 **
LCP – Biological (length)	0.775 **	0.778 **	0.774 **
LCP – Physical (cost)	0.758 **	0.753 **	0.760 **
LCP – Physical (length)	0.821 **	0.815**	0.825 **
LCP – Combined (cost)	0.522 **	0.523 **	0.494 **
LCP – Combined (length)	0.749 **	0.747 **	0.752 **
LCP – Binary (cost)	0.454 **	0.472 **	0.485 **
LCP – Binary (length)	0.458 **	0.476 **	0.489 **
IBR – Biological	0.378 **	0.336**	0.327 **
IBR – Physical	0.391 **	0.327 **	0.351 **
IBR – Combined	0.134	0.113	0.132
IBR – Binary	0.127	0.186 *	0.187 *

Table 3. Mantel correlation between pair-wise individual Euclidean distance, resistance distance (IBR), or least-cost distance (cost or length) and genetic distance ( $D_{PS}$  or  $a_r$ ). The Mantel test statistic  $r$  is based on a Pearson's product-moment correlation and significance values are based on 10,000 permutations. The binary habitat model isolated 86 individuals that were removed from the LCP and IBR models. \* significance at the  $p < 0.05$  level, \*\* significance at the  $p < 0.001$

<b>Spatial distance</b>	<b>Genetic distance</b>	
	$D_{PS}$	$a_r$
Euclidean distance	0.323 **	0.347 **
LCP – Biological (cost)	0.295 **	0.314 **
LCP – Biological (length)	0.309 **	0.328 **
LCP – Physical (cost)	0.302 **	0.328 **
LCP – Physical (length)	0.326 **	0.348 **
LCP – Combined (cost)	0.195 **	0.224 **
LCP – Combined (length)	0.302 **	0.317 **
LCP – Binary (cost)	0.261 **	0.258 **
LCP – Binary (length)	0.261 **	0.260 **
IBR – Biological	0.057 **	0.094 **
IBR – Physical	0.188 **	0.238 **
IBR – Combined	0.040 *	0.079 *
IBR - Binary	0.219 **	0.174 **

Table 4. Partial Mantel correlations between pair-wise population resistance distance (IBR), or least-cost distance (cost or length) and genetic distance ( $F_{ST}/(1-F_{ST})$ ,  $D_S$ ,  $D_{LR}$ ), while accounting for geographic distance. The Mantel test statistic  $r$  based on Pearson's product-moment correlation and significance values are based on 10000 permutations. The binary habitat model isolated 5 populations that were removed from the LCP and IBR models. \* significance at the  $p < 0.05$  level

Spatial distance	Genetic distance		
	$F_{ST}/(1-F_{ST})$	$D_S$	$D_{LR}$
LCP – Biological (cost)	0.056	0.107	-0.021
LCP – Biological (length)	-0.036	0.031	-0.016
LCP – Physical (cost)	-0.132	-0.107	-0.091
LCP – Physical (length)	0.118	0.146 ( $p = 0.06$ )	0.196 *
LCP – Combined (cost)	-0.052	-0.033	-0.107
LCP – Combined (length)	-0.002	0.028	0.0327
LCP – Binary (cost)	-0.058	0.015	0.007
LCP – Binary (length)	-0.064	0.012	0.003
IBR – Combined	-0.074	-0.107	-0.075
IBR – Biological	-0.136	-0.208	-0.234
IBR – Physical	-0.052	-0.170	-0.128
IBR - Binary	0.117	0.209 *	0.222 *

Table 5. Partial Mantel correlations between pair-wise individual resistance distance (IBR), or least-cost distance (cost or length) and genetic distance ( $D_{PS}$  or  $a_r$ ), while accounting for Euclidean distance. The partial Mantel test statistic  $r$  based on Pearson's product-moment correlation and significance values are based on 10000 permutations. The binary habitat model isolated 86 individuals that were removed from the LCP and IBR models. \* significance at the  $p < 0.05$  level, \*\* significance at the  $p < 0.001$

<b>Spatial distance</b>	<b>Genetic distance</b>	
	$D_{PS}$	$a_r$
LCP – Biological (cost)	0.064 **	0.063 **
LCP – Biological (length)	0.015	0.001
LCP – Physical (cost)	-0.044	0.009
LCP – Physical (length)	0.060 **	0.050 **
LCP – Combined (cost)	-0.003	0.016
LCP – Combined (length)	0.021 *	0.002
LCP – Binary (cost)	0.062 **	0.040 **
LCP – Binary (length)	0.060 **	0.039 **
IBR – Biological	0.011	0.046 *
IBR – Physical	-0.035	0.015
IBR – Combined	0.009	0.049 *
IBR - Binary	0.165 **	0.112 **

## FIGURE LEGENDS

Figure 1. Map of Mojave desert tortoises sampled for landscape genetics. Each colored icon represents an individual from one of nine genetic subpopulations (Virgin River = red, Muddy Mountains = light blue, Amargosa Desert = orange, South Las Vegas = dark blue, Eldorado Valley = teal, Piute Valley = purple, Northern Colorado = green, Eastern Colorado = yellow, Western Mojave = pink). Black lines represent interstate highways.

Figure 2. Distribution of desert tortoise habitat in the Mojave desert predicted using the GRASP model in Program R. Suitable habitat determined by (A) four biological variables: annual plant cover, mean dry (summer) season precipitation for 30-yr normal period, mean wet (winter) season precipitation for 30-yr normal period, and the spatially distributed coefficient of variation for wet season precipitation, (B) two physical variables: average surface roughness and elevation, (C) combined biological and physical model (six variables), and (D) binary habitat model in which suitable habitat was identified from the combined model using a threshold value. For A, B, and C gradient of colors (floating values) indicate probability of desert tortoise occurrence. Red indicates lowest probability (0) while blue indicates highest probability (1). In the binary model (D), grey indicates no habitat (0) and blue indicates habitat (1). Black stars represent 25 population centroids.

Figure 3. Cumulative least cost paths across 25 pair-wise population comparisons for different landscape variables (A) biological, (B) physical, (C) combined, and (D) binary. Varying shades of grey to black represent the habitat suitability model (black is not habitat, white is habitat). The red line indicates the least cost path. Blue dots represent 25 population centroids.

Figure 4. Cumulative current maps between pairs of populations from the isolation-by-resistance models using different landscape variables A) biological, (B) physical, (C) combined, and (D) binary. The gradient of colors (floating values) indicate probability of desert tortoise movement, with red regions indicating no current, yellow and orange regions representing low current, and blue regions representing high density current. Black stars represent 25 population centroids.

**FIGURES**

Figure 1.

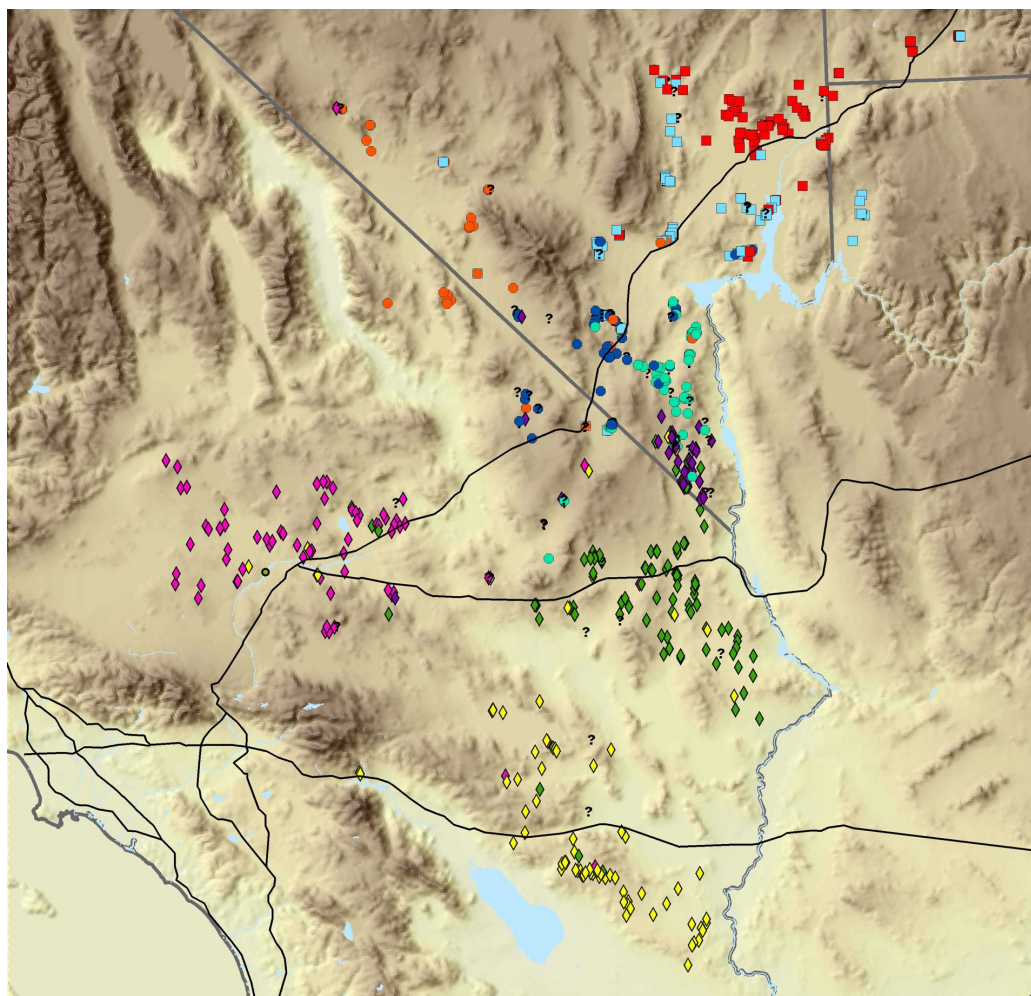


Figure 2.

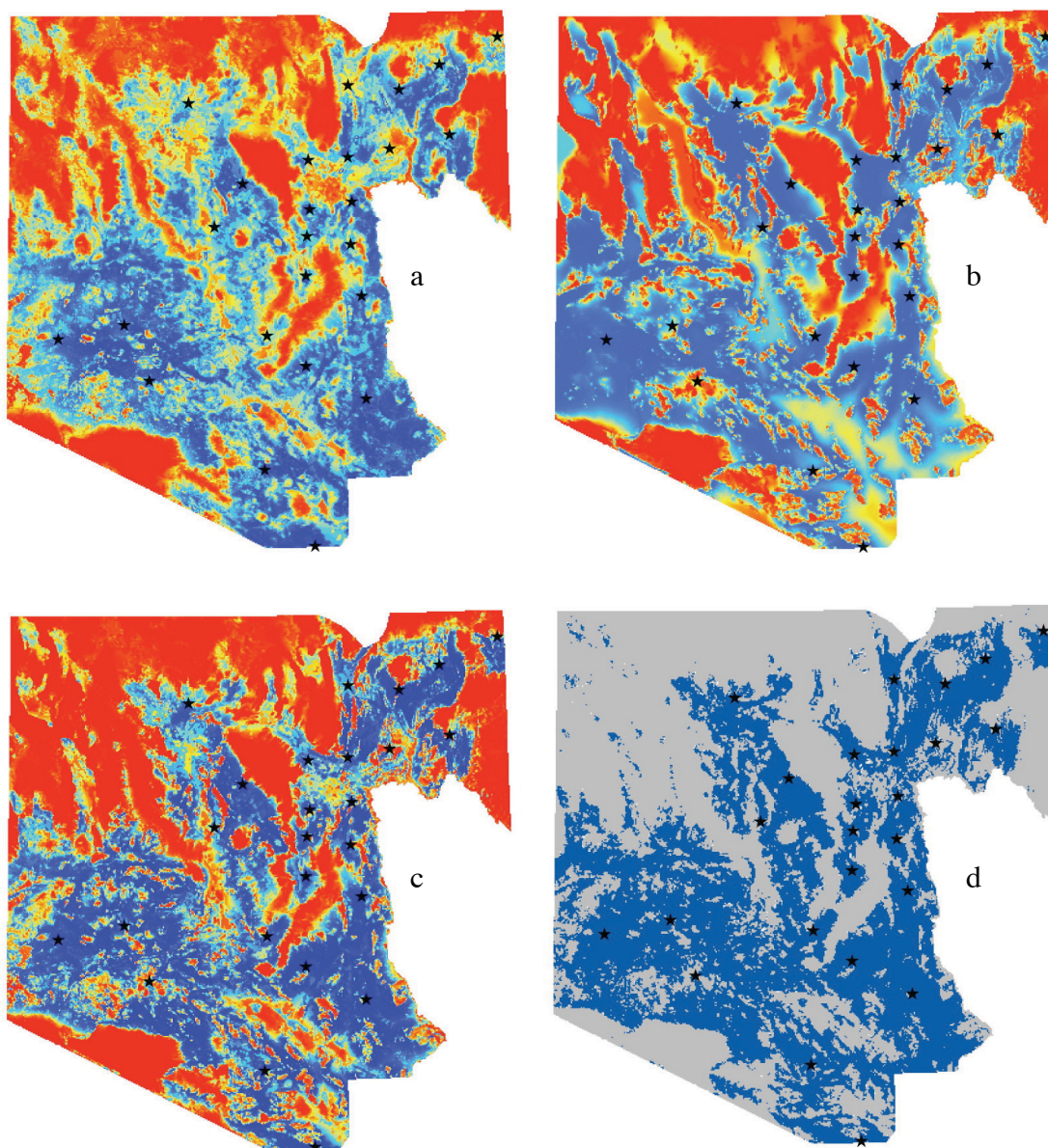


Figure 3.

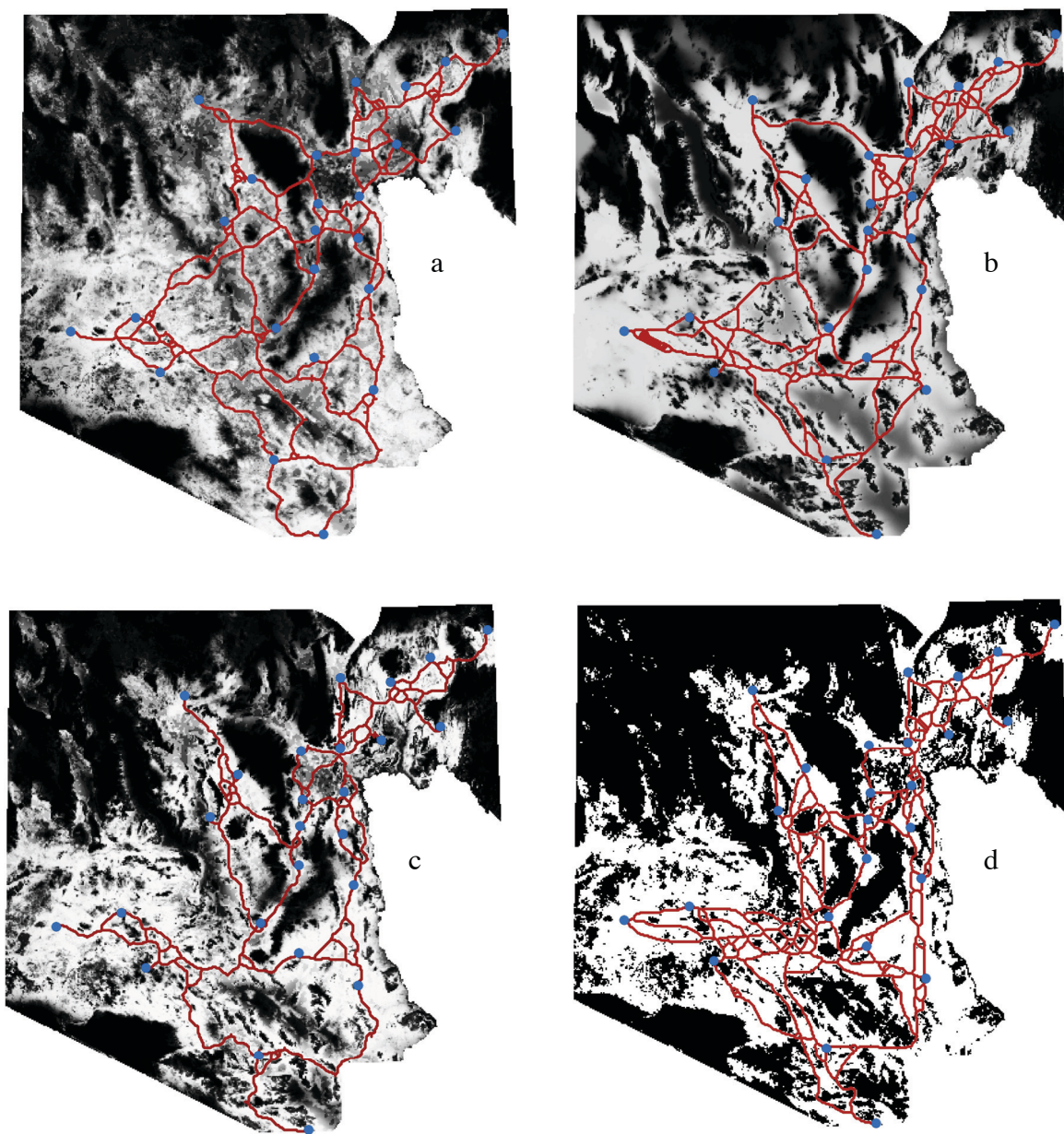
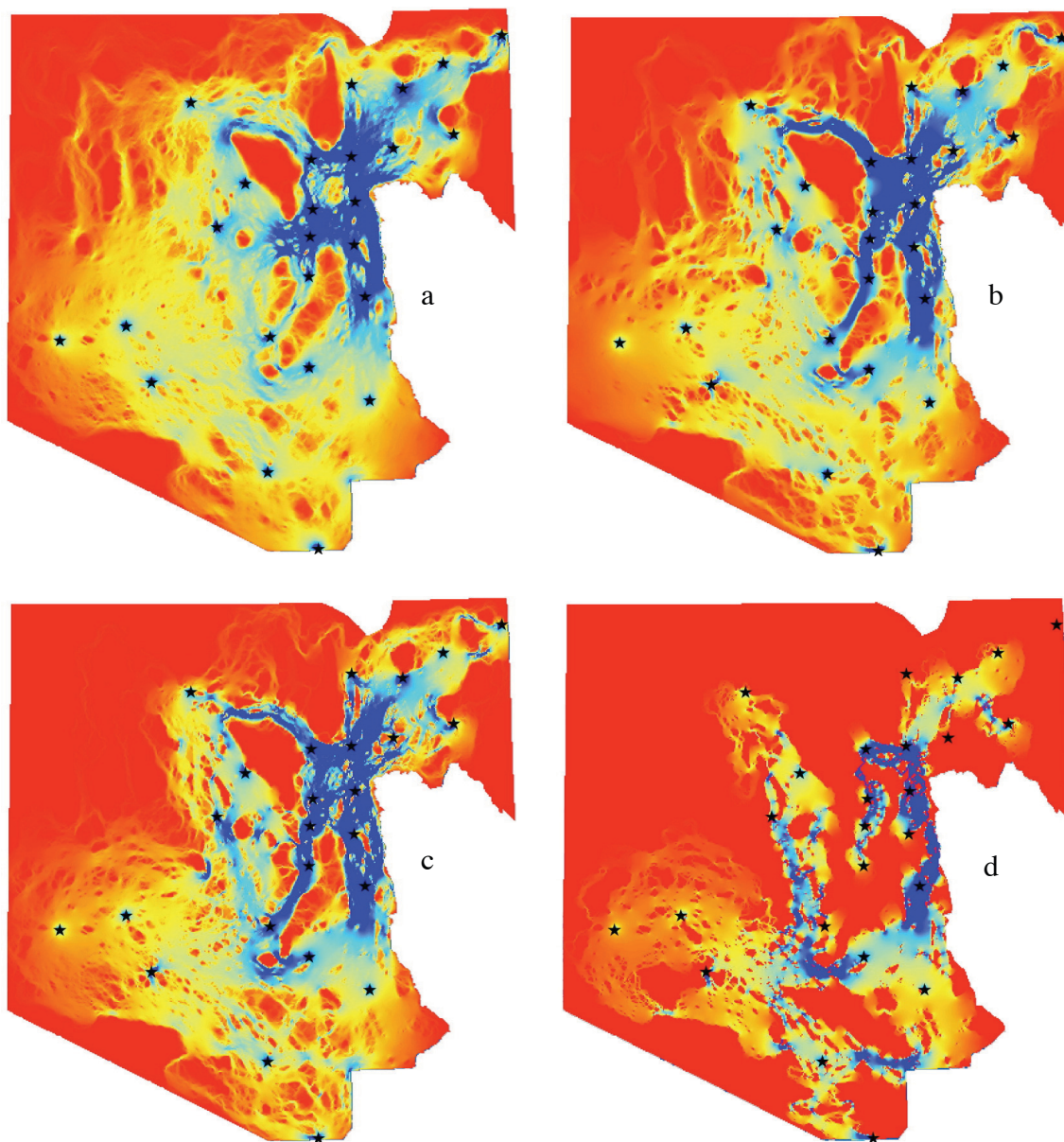


Figure 4.



### **CHAPTER 3. NEW INSIGHTS INTO CONSERVATION OF THE DESERT TORTOISE: IMPORTANCE OF GENETIC ANALYSES**

#### **ABSTRACT**

Genetic data from neutral markers are often used in conjunction with other population data to make critical decisions about species of conservation concern. Inferences from genetic data and analyses can be used to enhance and complement decision-making related to conservation units, reintroductions and translocations, habitat preservation and restoration, and mitigation of species-specific threats to population persistence. Here, we provide a case study for how the inferences made from highly-variable, neutral genetic markers (e.g., microsatellites) can complement other ecological data when making conservation decisions for a threatened species. We illustrate how population genetic data can be used in combination with other relevant biological data to make specific recommendations for the Mojave population of desert tortoise in each of the following areas. First, we provide evidence for revising the boundaries of recovery units based on genetic data as well as differences in ecology and behavior of desert tortoises that occur across environmental gradients in the Mojave Desert. Second, we show that individual-based assignment tests can determine the population of origin for Mojave desert tortoises, and these tests can provide guidance as to where individuals should be translocated. Finally, we explain why topographical data on the landscape scale improves our understanding of natural habitat corridors, and how analyses can be used to

inform management concerning maintaining levels of connectivity among desert tortoise subpopulations that are now fragmented by human land-use changes.

## OVERVIEW

To make important decisions about species of conservation concern, analyses using neutral genetic markers are often used in conjunction with other population data such as estimates of population size; estimates of demographic parameters such as survival, fecundity, and recruitment; and habitat requirements (DeSalle and Amato 2004, Hedrick 2004, Wayne and Morin 2004). Previously, general recommendations such as the maintenance of genetic variation and the prevention of inbreeding were the main contributions of population genetics to conservation biology (Hedrick and Morin 1992, Frankham 1995, Hedrick 2004). Beyond these general principles, neutral genetic markers can be used to answer many questions related to individuals and populations. For example, we can infer relatedness of individuals (Blouin et al. 1996), detect population bottlenecks (Cornuet and Luikart 1996), identify sex-biased dispersal (Favre et al. 1997), estimate gene flow (Paetkau et al. 2004), investigate parameters that mediate dispersal (Cushman et al. 2006), determine an individual's population of origin (Manel et al. 2003), and delineate population boundaries (Pritchard et al. 2000).

In turn, inferences from genetic analyses potentially can aid in decisions ranging from identifying conservation units to prioritizing habitat for restoration. The general benefits of conservation genetics, and the future directions in this field have been reviewed and are described thoroughly elsewhere (Hedrick 2001, Frankham et al. 2002, Moran 2002, Hedrick 2004, Wayne and Morin 2004). Here, we provide a case study for

how the inferences made from highly-variable, neutral genetic markers (e.g., microsatellites) can complement other ecological information to make conservation decisions for a threatened species.

The Mojave population of the desert tortoise (*Gopherus agassizii*), which was listed as threatened under the U.S. Endangered Species Act of 1973 (55 FR 12178, April 2, 1990), is in decline as a result of habitat destruction, invasive species, and many other threats related to increased human land use (Tracy et al. 2004, USFWS 1994, USFWS 2008). Desert tortoises are long-lived, have low growth rates and delayed sexual maturity (age 13-20), and have low annual reproductive rates over a long adult life span (Germano 1994). Reproduction and growth rates vary with heterogeneity in the harsh, environmental conditions of the Mojave Desert; individuals capitalize on rare years with high rainfall and productivity (Henen 1997). These traits cause tortoise populations to respond slowly to management actions, making evaluation of those actions difficult. Extreme and variable climatic conditions also reduce daily and seasonal activity of tortoises. Individuals spend a majority of each day and each season underground in burrows, making them elusive to human observers and researchers (Zimmerman et al. 1994). Additionally, the Mojave desert tortoise has a large geographic distribution, which extends across four states in the southwestern United states. Thus, management requires coordination among multiple federal, state, and local agencies, communities, and various non-governmental organizations to implement actions that are important to recovering the population.

The life history and ecological traits of this species and the complications of coordination across many political boundaries present unique challenges for

management. Inferences from genetic analyses complement other ecological information and can be used to improve conservation planning for this species. Additionally, these life history traits influence the temporal scale of our inferences. Our genetic data reflect ecological processes that were occurring prior to anthropogenic changes in the Mojave Desert. The recent effects of humans are not reflected in our data because there is a severe time lag caused by long generation times (Keyghobaldi 2007). This provides opportunities to make recommendations based upon population dynamics prior to severe human influences. We address three specific conservation topics important to recovering the Mojave population of the desert tortoise: (1) designation of conservation units, (2) translocation, and (3) maintenance of connectivity among subpopulations. Our goal is to use population genetic data and analyses in combination with other relevant biological data to make recommendations in each of these areas. We summarize inferences made from previous genetic studies and compare and contrast our results with those previous studies.

## **DESIGNATING CONSERVATION UNITS**

Delineating conservation or management units is a fundamental component of the management of natural populations (Palsboll et al. 2006). These units offer a framework with which to maintain and preserve intra-species diversity. Additionally, they provide a least common denominator for management, allowing managers to make decisions in local areas that will contribute to the overall conservation and management of a species. Generally, conservation units should represent specific regions that will be important to

conservation of the entire species or population. These units often have specific ecological, genetic, or behavioral attributes that separate them from other units, and/or these units also may vary in conservation status or the threats contributing to the declines. Conservation units are particularly important to wide-ranging species such as the desert tortoise. Within the listed portion of the range, tortoise habitat occurs on federal, state, military, and private land. Thus, multiple federal, state, local governments and agencies, non-governmental organizations, and private citizens can be accountable for implementation of management actions. The designation of appropriate conservation units for this species has several implications, including prioritization of habitat conservation and division of management responsibilities.

Generally, a species' recovery entails the removal of "threats" that are implicated in population declines. Eliminating these threats theoretically should allow populations to recover to targets set by a recovery plan. Under the U.S. Endangered Species Act, a required recovery plan outlines the specific criteria for evaluating recovery and the actions necessary to reach those goals for each species. Within the 1994 Recovery Plan for the Mojave desert tortoise, specific research needs included a description of population structure (USFWS 1994, Berry et al. 2002). Additionally, a recent assessment of the Recovery Plan recommended the collection of fine scale genetic data to enhance the delineation of conservation units (Tracy et al. 2004).

After the desert tortoise was listed as a threatened species, recovery units were designated as part of the recovery planning process. Recovery units should not be equated with distinct population segments, which are legally binding designations for listing portions of a species (e.g., the Mojave desert tortoise was listed as a distinct population

segment (55 FR 12178, April 2, 1990). Recovery units are not legally recognized in the U.S. Endangered Species Act, so they cannot be listed as their own entities, nor can they be individually removed from the list (NMFS 2006). Thus, all recovery units must meet the recovery criteria before the listed entity can be removed from the list of endangered species. Recently the U.S. Fish and Wildlife Service adopted a formal recovery unit policy (NMFS 2006). According to the policy, recovery units should be geographically identifiable and essential to the recovery of the Mojave population of the desert tortoise (NMFS 2006). Each unit should contain elements necessary to conserve genetic or demographic robustness, or elements required for the long-term sustainability of the whole distinct population segment, subpopulation, or species (NMFS 2006). In this discussion, we will offer suggestions for revising the recovery units for the desert tortoise. Recovery units could be considered conservation or management units more generally. We will continue to use the term recovery unit throughout our discussion, and other terms will be defined as needed.

The 1994 Recovery Plan for the Mojave desert tortoise identified six recovery units that encompassed the entire distribution of the listed distinct population segment of the Mojave desert tortoise: Upper Virgin River, Northeastern Mojave, Eastern Mojave, Eastern Colorado, Northern Colorado, and Western Mojave (USFWS 1994; Fig. 1). Critical habitat was identified within each original recovery unit (Desert Wildlife Management Areas or DWMAs) and remains vital for the recovery of the desert tortoise. These recovery units contained, genetic, morphological, ecological, and behavioral differences that were identified at a species-wide scale (Woodbury and Hardy 1948, Burge 1977, Jennings 1985, Turner et al. 1986, Weinstein and Berry 1987, Lamb et al.

1989, Glenn et al. 1990, Germano 1993, Lamb and Lydehard 1994, Wallis et al. 1999, Averill-Murray 2002, Averill-Murray et al. 2002a, Averill-Murray et al. 2002b).

Although finer-scale genetic, morphological, ecological, and behavioral differentiation was acknowledged within the Mojave population (USFWS 1994), the boundaries of the 1994 recovery units were poorly justified in some cases (Tracy et al. 2004). These units also did not reflect the qualifications for recovery units based on current policies (NFMS 2006) or more recent genetic and ecological data (Britten et al. 1997, Tracy et al. 2004, Murphy et al. 2007, Hagerty and Tracy in prep).

One definition of an evolutionarily significant unit, which describes a group below the level of species (Ryder 1986, Waples 1991, Mortiz 1994, Moritz 2002), was used as a guideline to identify the original recovery units. The evolutionarily significant unit differs from the distinct population segment because it is a biological definition and not a legal definition, except in the case of salmonids, where the two terms are synonymous (Peacock and Kirchoff 1995). Discussion and debate continues over a myriad of definitions for the evolutionarily significant unit, which range from strict quantitative criteria (Moritz 1994) to a more holistic, case-specific approach (Waples 1991, Crandal et al. 2000). Although these discussions have theoretical and practical implications, the management unit (Moritz 1994, Paetkau 1999, Palsboll et al. 2006) may be more appropriate as a conservation unit for the desert tortoise. Management units, which can be defined as populations with independent dynamics, are typically considered as less isolated than evolutionarily significant units and are useful for identifying local conservation and monitoring (Palsboll et al. 2006).

The following questions, which constitute a more holistic definition of the evolutionarily significant unit (given by Waples 1991) were used in the 1994 Recovery Plan and remain relevant for our discussion:

- (1) Is the population genetically distinct?
- (2) Does the population occupy unusual or distinct habitat?
- (3) Does the population show evidence of unusual or distinct adaptation to its environment?

These questions point to the additional purposes of a recovery unit besides the need to protect the genetic diversity and evolutionary potential of the listed population. Specifically, recovery units are seen as important to protect the listed population by providing the ecosystem protection needed to preserve ecological interactions required of a recovering population. Diversity of food and shelter resources, as well as potential variation in host/pathogen and predator/prey interactions, must be incorporated in recovery units to ensure the species ability to avoid demographic and stochastic threats to persistence including climate change. Therefore, recovery units must protect unique habitats and unique ecological interactions including those with humans.

Within this definition, multiple forms of evidence can be used to delineate justifiable recovery units for the desert tortoise. Genetic and morphological data are commonly used to distinguish among populations or groups of populations that are significantly different and contribute to the diversity that defines the species or distinct population segment. Details of natural history and distribution of the species can be valuable for describing these units, particularly when neutral genetic markers, which do

not provide direct information on natural selection, are used to make initial distinctions (Green 2005).

We propose new delineations of conservation units for the Mojave desert tortoise, which could be used to identify recovery units for this population. Our approach includes genetic, evolutionary, and ecological considerations. We propose eight recovery units that represent the most parsimonious hypothesis, however, this configuration of units is only one of several tenable hypotheses for assigning units. Additional genetic, demographic, and ecological data should be used to test and revise this hypothesis. The entire distribution of the threatened range is covered by our proposal, and all critical habitat areas remain well justified. Additional areas of critical habitat may be required, and these recommendations are discussed below.

Genetic differentiation, geography, climatic and ecological differences in diverse vegetation types, as well as characteristics of desert tortoise biology can be used to support the boundaries suggested for the eight recovery units. Subpopulations of the desert tortoise within the Mojave and Colorado deserts are not highly genetically differentiated ( $F_{ST} = 0.012$  to  $0.132$ ), particularly when levels of differentiation are compared to other species that have widespread distributions (Hagerty and Tracy in prep). However, the extensive distribution of the Mojave desert tortoise, as well as complex topographic relief across the range, contributes to a significant gradient in allele frequencies between the most northeast and southwest corners of the range (Hagerty and Tracy in prep). Furthermore, various natural boundaries with varying degrees of permeability reinforce the isolation of subpopulations caused solely by geographic distance (Hagerty et al. in prep). Variation in available habitat translates into potentially

different selective pressures for populations of the desert tortoise. Widespread differences in activity patterns, foraging behavior, and life history characteristics (driven by geography, climate, and plant communities) are used to support recovery units.

The backbone for the eight proposed recovery units is genetic differentiation described by 20 hyper-variable genetic markers (Hagerty and Tracy in prep). We identified seven genetic subpopulations based upon individual-based assignment tests that mainly rely on differences in allele frequencies among groups (Hagerty and Tracy in prep). These subpopulations are: Virgin River, Muddy Mountains, South Las Vegas, Amargosa Desert, Northern Colorado, Eastern Colorado, and Western Mojave (Fig. 2). Our methods did not require a priori definitions of where demes occurred (i.e., Bayesian assignment tests, Pritchard et al. 2000, Guillot et al. 2005), which enhanced our ability to identify population boundaries. In addition, we sampled as uniformly and extensively as possible across the listed range of the species which is necessary to make inferences about population boundaries (Manel et al 2003, Storfer et al. 2007).

The revised boundaries for the recovery units that we describe differ from those described in the 1994 Recovery Plan, with most differences occurring in Nevada. The northern, western, and southern boundaries on the periphery of the recovery units are defined by the distributional limits of the desert tortoise; the Colorado River forms a complete physical barrier in the east (Fig. 3). Differences from the original recovery units occur within those major range limitations. We identified four subpopulations within the original Northeastern Mojave Recover Unit: Lower Virgin River, Muddy Mountains, South Las Vegas, and Amargosa Desert (Fig. 3). These modifications support a previous

hypothesis stating that the original recovery unit contained additional genetic diversity (Britten et al. 1997).

The Amargosa Desert Recovery Unit contains a previously undescribed genetic subpopulation. Inadequate sampling in this area obviated its inclusion in early genetic analyses and caused tortoises in this area to be overlooked. Importantly, this recovery unit is genetically distinct from neighboring units, bordered by Death Valley on the west and the Spring Mountains in the east (Hagerty and Tracy in prep). Both of these ecological boundaries are formidable for tortoises, particularly the extreme thermal environment in Death Valley. This new recovery unit lacks critical habitat designations in Nye County, Nevada, where the majority of the land area for this unit occurs (Fig. 2 and Fig. 3).

Boundaries for the three recovery units in California remain almost identical to the descriptions in the 1994 Recovery Plan. The Northern Colorado recovery unit, which also contains Piute Valley, borders the South Las Vegas recovery unit. The boundary occurs at Searchlight Pass, which is a low elevation pass (1500 m). The Eastern Colorado recovery unit represents the most southern extent of the listed population. A low-elevation barrier, known as the Baker Sink, extends from Saline Valley in California in the north, then south through Death Valley, Silurian Valley, Baker, Amboy, and Cadiz Valley. This barrier separates the Northern and Eastern Colorado and reflects the formidable effects of the lower elevations and extremely hot climates along this line. The Western Mojave cluster is separated from the Eastern Colorado cluster in the Pinto Mountains, and from the Amargosa cluster in the low elevation area near Death Valley.

Although population genetics are a good foundation for delineating conservation units, genetics information alone does not clearly indicate unique evolutionary potential or adaptive differences (Paetkau 1999, Taylor and Dizon 1999, Green 2005, Palsboll et al. 2006). Additional information can be used to support delineations. For example, differences in vegetation and climate tend to shape differences in foraging ecology, habitat use, and life history traits of tortoises in each recovery unit (Peterson 1994, USFWS 1994, Peterson 1996, Henen et al. 1998, Lovich et al. 1999, Wallis et al. 1999, Tracy et al. 2004). Many ecological differences in the Mojave and Colorado Deserts, including climate and vegetation, also occur along gradients. These deserts are transitional ecosystems between the Great Basin and Sonoran Deserts (Rowlands et al. 1982, Turner 1982, MacMahon 1990). Therefore, we delineated boundaries with evident geographic barriers, such as mountain ranges and hot and dry low elevation playas (Hagerty and Tracy in prep).

Although tortoises in the northeastern extreme edge of the range near St. George, Utah were genetically similar to these adjacent locations in Nevada, morphological, ecological, and behavioral data distinguish those individuals. Therefore, we characterized the Upper Virgin River as the eighth recovery unit, which reflects the original recovery unit designation (USFWS 1994). The tortoises in the Upper Virgin River Recovery Unit represent the northern-most extent of the distribution of this species. Desert tortoises in this region experience long, cold winters (about 100 freezing days per year) and mild summers, during which the tortoises are active without a period of torpor due to stressfully hot summer conditions as can be seen in other parts of the species range (Woodbury and Hardy 1948). Here, tortoises live in a complex topography consisting of

canyons, mesas, sand dunes, and sandstone outcrops where the vegetation is a transitional mixture of Great Basin and Mojave vegetational associations (USFWS 1994). Desert tortoises use natural caves in sandstone and lava instead of burrows excavated by the tortoises, and two or more desert tortoises often use the same burrow (Woodbury and Hardy 1948, Esque 1994). Tortoises also travel to sand dunes to lay eggs and use other habitats for foraging (Woodbury and Hardy 1948). The unique habitat and resulting behavioral differences in tortoises in this region warrant explicit protection.

West of the Baker Sink, the ecological western Mojave Desert has variable rainfall typically occurring in the winter and spring and almost never as summer monsoonal rains. The resulting vegetation is quite different from eastern areas in the Mojave Desert (Germano et al. 1994, Tracy et al. 2004). Less variable winter rains as well as summer monsoon precipitation characterize the ecological Eastern Mojave Desert (Germano et al. 1994, Tracy et al. 2004). The most noticeable differences in tortoise ecology result from this climatic gradient between the western and eastern Mojave Desert (Tracy et al. 2004). Tortoises in the Western Mojave Recovery Unit produce relatively larger eggs, produce fewer eggs overall, and lay their second clutches later than do tortoises in the adjacent eastern Mojave Desert (Wallis et al. 1999). Behaviorally, Western Mojave tortoises are much less active during summer than are tortoises in other proposed recovery units (Marlow 1979, Nagy and Medica 1986).

As a distinct biome, the Colorado Desert contains a unique combination of Sonoran Desert and Mojave Desert flora (Burk 1977). While many plant species can be found in both of these two deserts, the Colorado Desert contains some arboreal species that are sensitive to freezing (Burk 1977, Lovich and Bainbridge 1999). Reliable

precipitation in the summer in the Colorado Desert results from the monsoonal influence of the Sonoran Desert and provides two distinct periods with different sets of annual flora available to tortoises. The more mild winters in the Eastern Colorado with fewer freezing days per year allow tortoises to burrow shallowly under shrubs and in washes. In the Colorado Desert, female tortoises produce additional clutches (up to three) in one reproductive season (Lovich pers. comm.), and produce smaller eggs than similarly sized females in the northeastern Mojave Desert near the Nevada Test Site (Mueller et al. 1998). The Baker Sink, a low elevation area that is hot and arid, divides both the western and eastern Mojave Desert and the Northern and Eastern Colorado recovery units. Although the Baker Sink may not be a complete barrier to tortoise movement, the extremely high temperatures and lack of shelter sites reduces the ability of individuals to move among subpopulations, and hence, recovery units.

## **TRANSLOCATION AS A MANAGEMENT TOOL**

Many conservation plans include translocation as a means to augment or reestablish populations or to establish new populations (Griffiths et al. 1989, Fischer and Lindenmayer 2000), or to prevent harm to individuals of a species affected by human activities (Edgar et al. 2005). Translocations have had varying levels of success depending upon the taxon and the circumstances under which the translocations occurred (Griffiths et al. 1989, Fischer and Lindenmayer 2000). To determine how to use translocation as a conservation tool, widely accepted criteria for a successful translocation must be established (Fischer and Lindenmayer 2000, Seigel and Dodd

2002). Additionally, translocation should always be regarded as an experiment requiring data from long-term monitoring to evaluate the effects of translocations on populations (Fischer and Lindenmayer 2000, Seigel and Dodd 2002, Field et al. 2006). Although the success of translocations is unclear in some cases, managers often have few options when populations have been extirpated from all or part of their native range (Nelson et al 2002). Therefore, careful planning of translocations is imperative to increase the probability that they will result in high survival, reproduction, and self-sustaining populations. Considering welfare, habitat requirements, disease, behavior, mating system, and genetics prior to releasing animals is critical because translocations can affect both residents and translocated individuals (Griffiths et al. 1989, Tracy et al. 2004, Teixeira et al. 2007).

The very high rate of human population growth in the Mojave Desert and the resulting habitat destruction has been causing continual displacement of tortoises in the threatened population. Currently, managers implement 'rescue' translocations, which remove tortoises from sites scheduled for urban development, or from currently urbanized areas (see Edgar et al. 2005). In Clark County in Nevada, and Washington County in Utah, tortoises are removed from natural habitats prior to urban development and they are transferred to a holding facility. In Clark County, tortoises that do not test positive for antibodies to *Mycoplasma agassizii* (a pathogen implicated in causing Upper Respiratory Tract Disease) are relocated to a large fenced area southwest of Las Vegas. Translocations also have been conducted on military lands, such as The National Training Center at Fort Irwin, which is expanding its training area and converting desert tortoise habitat to military tank training sites (Esque et al. 2005, Heaton et al. 2008).

In addition to the rescue translocations, this management tool may be necessary to reestablish or bolster populations that have dwindled or have been extirpated. Due to the potential importance of this conservation action, the 1994 Recovery Plan for the Mojave desert tortoise included guidelines to determine the effectiveness of translocations (USFWS 1994). Recently conducted experiments were designed to test the feasibility of translocating desert tortoises (Nussear 2004, Field et al. 2006). In previous studies, translocated tortoises were at higher risk from predation, from harsh abiotic conditions that could cause (among other things) overheating, and from an inability to find shelter and forage sites (Berry 1974, Cook et al. 1978, Berry 1986). However, tortoises in the cited studies were moved during extremely hot periods when tortoises would likely experience lethally high temperatures. When administered properly, translocation appears to be an effective tool for minimizing the impacts of development on tortoises and augmenting wild populations (Nussear 2004, Field et al. 2006). In the short term, translocated tortoises showed similar mortality and reproductive rates, developed similar activity and movement patterns after an initial adjustment period, and the translocation had no effect on the survival, movement or reproduction of resident animals (Nussear 2004, Field et al. 2006). Other impacts, such as potential physiological stress, and long-term impacts of translocation and sustainability of augmented populations are currently being investigated experimentally (Esque et al. 2005).

The genetic consequences of translocation are not well understood and most studies focus on reintroductions (individuals introduced to areas not containing individuals of the same species), which are sensitive to the number of individuals released and their genetic diversity (Stockwell et al. 1996, Vinkley et al 2006). If a

population is reestablished with too few founders, genetic diversity may be low and the reduced number of founding individuals could cause a population bottleneck (Cornuet and Luikart 1996). Additionally, if individuals are introduced to a population of residents that are too genetically divergent, outbreeding depression could occur, disrupting local adaptation (Tallmon et al. 2004).

Population genetics tools also can be used to evaluate genetic diversity in established populations, or to determine the population of origin for displaced individuals. Genetic analyses could be used to evaluate the relative contribution of the genes of translocated animals to the recipient population, but the ability to detect those differences would depend upon demonstrable genetic differences between the donor and recipient populations. Additionally, the genetic signature of translocation is not likely to be visible in the short term and would require many generations before the genetic signature is discernable. Population genetics can be helpful in ‘rescue’ translocations (moving animals from an area of threat to the tortoises to an area where there is an existing population) by providing data on the genetic difference between the donor and recipient populations, and information about where displaced individuals should be relocated.

As a case study, we used individual-based assignment tests to evaluate how effectively individual desert tortoises from a resident population in Clark County, Nevada and individuals translocated into the area could be traced back to their population of origin. In Clark County, tortoises are relocated to the Large Scale Translocation Site (LSTS), which is a 90 km<sup>2</sup> area located between the town of Jean, Nevada and the California/Nevada border, and bordered in the east by Interstate Highway 15. The site is

fenced on three sides to prevent movement of tortoises, and the Spring Mountains act as a border to the west. Before translocation began in spring 1997, the resident population density in the LSTS was approximately 20-25 tortoises/km<sup>2</sup>. Since 1997 approximately 6,000 desert tortoises from varying age classes have been released into the site (Southern Nevada Environmental, Inc. pers. comm.).

Desert tortoises that are translocated into the LSTS potentially originate from any of several subpopulations within the Mojave Desert, particularly because many tortoises removed from Las Vegas Valley have been captive (pets) at one time. If translocated individuals originate from genetically different subpopulations, these individuals and their offspring could change the genetic signature of the recipient population. However, desert tortoises exhibit low-to-moderate genetic differentiation, and they are structured mainly by isolation-by-distance (Hagerty and Tracy in prep). Therefore, subpopulations that are separated by considerable geographic distances (> 200 km) are more likely to exhibit significantly different allele frequencies (if the method by which one assesses genetic differences is sufficiently discerning).

We collected blood samples from translocated individuals (n = 23) and resident individuals (n = 25) inside the LSTS (Translocated individuals were identified by numbers adhered to the carapace with epoxy while the tortoises were housed at the Desert Tortoise Conservation Center). Samples were collected on randomly-placed 12 km transects in conjunction with training for the routine USFWS population monitoring (USFWS 2006) or opportunistically while traveling to or from the assigned transect.

Our laboratory procedures followed those described in Hagerty and Tracy (in prep). The 20 microsatellites used in this study included loci originally developed for

*Gopherus polyphemus* (Schwartz et al. 2003) and the Sonoran population of *Gopherus agassizii* (Edwards et al. 2003), as well as loci developed specifically for the Mojave desert tortoise (Hagerty et al. 2008). We amplified microsatellites and completed fragment analysis in collaboration with the Nevada Genomics Center (<http://www.ag.unr.edu/Genomics/>). All alleles were scored with GeneMapper 5.0 (Applied Biosystems, Inc.).

Individuals were assigned probabilistically to one of nine genetic subpopulations that were identified previously (Hagerty and Tracy in prep) using GENECLASS 2.0 (Piry et al. 2004). The LSTS is located within one of those subpopulations; the South Las Vegas subpopulation. A data set including 748 tortoise genotypes was used as the reference to assign the resident and translocated individuals (Hagerty and Tracy in prep), which we treated as originating from an unknown location. We used a frequency-based approach to calculate the genotype likelihoods (Paetkau et al. 1995). Significance testing ( $\alpha = 0.01$ ) was implemented using a Monte Carlo re-sampling algorithm. Multilocus gametes with replacement were drawn from randomly chosen individuals to simulate a data set of 10,000 individuals for each subpopulation (Paetkau et al. 2004). Genotype likelihoods that surpassed the critical value were assigned to one of the nine subpopulations. If an individual's genotype could be assigned to more than one population, the subpopulation with the highest probability surpassing the critical value was used.

76% of individuals sampled as residents in LSTS were assigned to the South Las Vegas subpopulation. Only one individual was not assigned because its genotype likelihood did not surpass the critical value. The remaining 20% of individuals (5/25)

were assigned to one of the adjacent subpopulations, either Amargosa Desert or Eldorado Valley (Table 1). In comparison, 56.5% of the translocated individuals were assigned to South Las Vegas. Two of 23 individuals could not be assigned to any population because they did not meet the probability threshold. 35% of the individuals were assigned to one of four other subpopulations, Muddy Mountains, Amargosa Desert, Eldorado Valley, or Piute Valley (Table 2). No individuals from either group were assigned to any California subpopulation (Northern Colorado, Eastern Colorado, Western Mojave) or to the Virgin River population (the most northern extent of the desert tortoise's range).

Although we were able to correctly assign approximately 75% of the residents from LSTS to the South Las Vegas subpopulation, there was still a fair percentage that assigned to adjacent populations. It is first important to recognize that there were potentially high levels of gene flow among adjacent locations, so differences among subpopulations with neighboring borders are small. Second, the difference between Eldorado Valley, Piute Valley and their adjacent locales was obscure in a previous study because uneven and high sample sizes in that region appeared to cause a spurious genotype cluster (Hagerty and Tracy in prep). When sample sizes were systematically reduced, the Eldorado Valley subpopulation became part of the South Las Vegas Cluster (Hagerty and Tracy in prep). If those individuals were included in the correctly assigned group, self-assignment increased to 88%. Low genetic differentiation between the Amargosa Desert and South Las Vegas subpopulations ( $F_{ST} = 0.012$ ) could account for the incorrect assignments.

Assignment of translocated individuals to the South Las Vegas subpopulation was lower than for residents. Tortoises that are housed temporarily at the Desert Tortoise

Conservation Center can originate from different regions of Las Vegas Valley, which itself is an interesting location because three subpopulations connect in the Las Vegas Valley (Hagerty and Tracy in prep). The northeastern corner of Las Vegas Valley is part of the Muddy Mountains subpopulation, and the northwestern portion of the valley is associated with the Amargosa Desert subpopulation. Although most tortoises at the DTCC were taken from habitat slated for urban development, a proportion of those animals were captive (pet) tortoises, which have unknown origins. One translocated tortoise assigned to Piute Valley, which clusters genetically with the Northern Colorado subpopulations in California. Piute Valley is twice as genetically different from South Las Vegas ( $F_{ST} = 0.029$ ) than is Eldorado Valley ( $F_{ST} = 0.014$ ; Hagerty and Tracy in prep).

Our study indicates that we can assign unknown individuals in the Mojave population to their likely subpopulation of origin with some degree of certainty. However, these subpopulations cover large geographic areas and gene flow among them has been historically high as evidenced by low levels of differentiation. Therefore, boundaries do not create discrete subpopulations, making assignment to an original location more difficult. Potentially rare alleles found in some individuals of localized areas can be used to support or clarify results of assignment tests. For example, one of the two translocated individuals that was not assigned contained a private allele (at locus GOA14) which is found in individuals from the South Las Vegas Cluster, suggesting that this particular individual originated from the South Las Vegas area. In total, we identified 36 private alleles across all nine subpopulations, with only the Amargosa Desert not containing any private allele (Table 3). Assignment tests and private alleles can be useful

tools for determining the home subpopulation for tortoises of unknown origin and these tools can provide guidance for where individuals should be translocated.

The small sample of translocated tortoises genotyped for this study contained one individual that was assigned to a genetically differentiated subpopulation (Piute Valley) and several other individuals that were assigned to adjacent populations. The presence of this individual among the translocated group suggests that translocated individuals could alter the genetic signature of the resident population in LSTS. As mentioned previously, gene flow has occurred historically among subpopulations; however, managers should take precautions to ensure that movement of individuals will maintain the historic population structure dominated by isolation by distance. Genotyping individuals prior to movement to a new location may be necessary to prevent translocations from causing panmixia or outbreeding depression.

## **MAINTAINING CONNECTIVITY AMONG POPULATIONS**

Connections among populations and habitats are necessary to maintain ecological processes such as dispersal and outbreeding, which allow populations and species to persist (Crooks and Sanjayan 2006). As habitat fragmentation increases subdivision and isolation of many populations, these ecosystem processes are disrupted (Gilpin and Hanski 1991). Natural population dynamics can be altered by reduction or removal of dispersal and gene flow. Demographic and environmental stochasticity will tend to increase, edge effects will increase, genetic diversity is predicted to decline, and ultimately fragmentation could lead to population extirpation (Saunders et al. 2001,

Fischer and Lindenmayer 2007). Therefore, finding potential solutions to restore linkages among populations and habitats can be a conservation priority (Crooks and Sanjayan 2006).

Landscape connectivity combines the structure of the landscape, which includes area, shape, and location of landscape features, with the response of individuals to those relevant components of the landscape (Brooks 2003, Taylor et al. 2006). Therefore, connectivity does not have a universal meaning for all organisms and depends upon the appropriate spatial and temporal scale for each species and its habitat (Holdregger and Wagner 2008). Describing the structural component of landscape that each species experiences is feasible due to the advent of Geographic Information Systems (GIS). However, successful measurements of the behavioral response to landscape features involve actual movement of individuals and their genes (Brooks 2003). Estimates of gene flow provide a cumulative signature of the movement of individuals that results in reproduction or permanent immigration and do not include unsuccessful movements (Whitlock and McCauley 1999, Mech and Hallett 2001, Brooks 2003). Further, genetic techniques can be logistically less complicated than direct field efforts (Koenig et al. 1996, Mech and Hallett 2001), though a combination of these methods seems most desirable.

The genetic effects of habitat fragmentation may not be visible in species like the desert tortoise because the temporal scale at which patterns of gene flow change is much slower than the scale at which land use changes (Varvio et al. 1986, Keyghobaldi 2007). The size of the population, and generation time (affected by life span and age of sexual maturity) affects the rate genetic changes will occur in response to isolation (Varvio et al.

1986, Paetkau 1999). In many cases, many hundreds of years would be necessary to detect negative consequences of fragmentation and reduction in dispersal (Varvio et al. 1986, Cushman et al. 2006, Keyghobaldi 2007, but see Gerlach and Musolf 2000, Epps et al. 2005, Proctor et al. 2005). Therefore, the temporal lag associated with the genetic effects of habitat fragmentation is an important point when trying to detect differences in isolated habitats.

Temporal lag in genetic signatures of habitat fragmentation also offers a unique opportunity to evaluate population structure and connectivity prior to major anthropogenic changes. Because the desert tortoise is a long-lived species with late age of sexual maturity it is less possible to detect genetic effects of urbanization and other human impacts fragmenting the Mojave Desert. While major changes have occurred across the Mojave Desert in the past century (Lovich and Bainbridge 1999, Hunter et al. 2003), a century translates into only four desert tortoise generations (estimated as 25 years; USFWS 1994). Thus, evaluating population structure and connectivity using genetic data allows us to make inferences about habitat fragmentation on the tortoise populations and to provide recommendations to sustain historic population dynamics. Here, we use evidence from microsatellite genetic markers to infer that desert tortoise subpopulations were connected in the past by high levels of gene flow. Additionally, we discuss factors mediating gene flow and present an argument that gene flow is not currently occurring due to urbanization and the transportation infrastructure in the southwestern United States. Finally, we suggest some potential management actions to maintain gene flow among subpopulations.

Within the Mojave population of the desert tortoise, subpopulations have low to moderate levels of genetic differentiation (Hagerty and Tracy in prep, Murphy et al. 2007). These small differences in allele frequencies ( $F_{ST} = 0.012 - 0.132$ , Hagerty and Tracy in prep) could indicate low levels of current gene flow and/or modest levels of historic gene flow (Keyghobaldi 2007). Unfortunately, several ecological processes shape inferences made about genetic structure using classical population genetic approaches, and genetic structure can be influenced by the effective size of populations (Bossart and Prowell 1998). Differentiation among tortoise locations occurs along a gradient indicating a continuous population in a way leading to genetic structure being highly correlated to geographic distance. Indeed, geographic distance explains approximately 65% of the variation in genetic distances among sampling locations, suggesting that the desert tortoise conforms to an isolation-by-distances model (Murphy et al. 2007, Hagerty and Tracy in prep.).

Estimates of the number of migrants per generation ( $Nm$ ) calculated from  $F_{ST}$  values are potentially misleading, thus we chose to use an individual-based assignment method to identify probable first generation migrants (Paetkau et al. 2004, Piry et al. 2004). To identify migrants, we used a data set consisting of 748 Mojave desert tortoises sampled from across the range and genotyped at 20 microsatellite loci (Hagerty et al. 2008, Hagerty and Tracy in prep.). We assigned the individuals to one of nine subpopulations (Hagerty and Tracy in prep.) that were identified using Bayesian clustering methods (Pritchard et al. 2000, Guillot et al. 2005). We identified first generation migrants using the likelihood ratio ( $L_{HOME}/L_{MAX}$ ) with an assignment criteria based upon allele frequencies (Paetkau et al. 1995).

We identified 34 individuals as migrants (individuals found outside of their home subpopulation) with a probability of error less than 0.01 (Table 4). However, we evaluated the genetic distance ( $D_{LR}$ ) and plotted genotype likelihoods between pairs of subpopulations to determine the extent to which we had sufficient power to detect migrants (as suggested by Paetkau et al. 2004; also see Proctor et al. 2005). When pairs of populations had genetic distances below three ( $D_{LR} < 3$ ) and assignment plots did not show separation of those populations, we did not count them as migrants. We did not have the power to detect migrants among some of the geographically-adjacent subpopulations, and the total number of migrants was reduced to 22 individuals (Table 4). These migrants are neither likely to be from the previous generation due to the time lag associated with these genetic data, nor do they give an exact estimate of the number of successful dispersal events in one generation. However, the pattern of assignment supports geographic distance as an indicator of genetic structure and suggests that gene flow was high among subpopulations at least in the recent past.

#### Habitat Fragmentation and Bottlenecking

Fragmented populations tend to decrease in size, which can increase the effects of genetic drift and result in a loss of genetic diversity (Gilpin and Hanski 1991, Keyghobaldi 2007). Additionally, population bottlenecks may occur, which also can provide a genetic signature, depending on the time since the bottleneck event and its severity (Andersen et al. 2004). To investigate the potential of population bottlenecks in the recent past (described in Hagerty and Tracy in prep), we used two analyses, which

predict different genetic consequences of a reduction in population size. The first assesses a reduction in the effective population size, as a lower number of alleles than would be predicted for the observed heterozygosity (allele deficiency and excess heterozygosity) (Cornuet and Luikart 1996). We calculated the distribution of gene diversity expected under the assumption of mutation-drift equilibrium and a two-phase mutation model for microsatellites (Di Rienzo et al. 1994, Luikart et al. 1998) in Program BOTTLENECK. Expected gene diversity was compared to the observed gene diversity to evaluate the extent to which an excess or deficit of heterozygosity exists. We used the Wilcoxon test for statistical significance as it provides high power with more than 15 individuals and 10-15 microsatellites (Luikart et al. 1997).

The second potential consequence of a bottleneck is a larger reduction in the number of alleles compared to the range in allele size in a population. Thus, we also tested for population bottlenecks in the 25 sampling locations using the *M*-ratio (Garza and Williamson 2001). The *M*-Ratio is determined by the equation  $M = k / r$ , where *k* is the total number of alleles and *r* is the overall range in allele size. *M* is expected to be smaller in populations that have recently experienced a reduction in population size. Significance of the observed *M* value is calculated by comparing it to an *M* value expected under equilibrium conditions using a two-phase mutation model (Di Rienzo et al. 1994). With more than seven loci and 25 or more individuals, a population probably experienced a bottleneck if  $M < 0.68$  (Garza and Williamson 2001).

Only the geographic sampling region in the South-I-15 corridor along the Nevada/California border had evidence of a population bottleneck using the Wilcoxon test in BOTTLENECK (Table 5). All other locations showed no indication of a

bottleneck using excess heterozygosity as an indicator. The South 1-15 corridor also had a low M-ratio value, close to 0.68, which is also an indicator of a bottleneck (Table 5). Although other locations had low M-ratios (Table 5), these locations had fewer than 20 individuals sampled, making the M values unreliable (Garza and Williamson 2001). The lack of genetic evidence for population bottlenecks provides support that subpopulations were not isolated and that they did not experience a reduction in population size that would cause a genetic signature. However, the time lag in these genetic data would prevent our detection of a bottleneck caused by human actions within the past century or longer.

#### Habitat Features Influencing Tortoise Movements

Finally, we recently used a landscape genetics approach to identify structural components in the landscape that can shape movement patterns of desert tortoises (Manel et al. 2003, Storfer et al. 2007, Holdregger and Wagner 2008). When a population has a continuous distribution, isolation-by-distance can be a null model of how patterns of genetic differentiation arise in the absence of barriers (Epperson 2003, Manel et al. 2003). Although geographic distances among desert tortoise subpopulations is strongly correlated with genetic distance, we expected that certain topographical features, such as large mountain ranges, would influence patterns of gene flow (Hagerty et al. in prep).

We tested multiple models, which altered the straight-line distances by accounting for the cost of movement through the landscape based on biological and physical variables (least cost path, Adriaensen et al. 2003, Theobald 2006; isolation by resistance,

McCrae 2006, McCrae and Beier 2007). Previously these variables were used to predict successfully desert tortoise occurrence in the Mojave Desert (Thomas et al. in review). Our models provided evidence that certain variables, specifically elevation and average surface roughness, indeed influence gene flow and hence historical dispersal of desert tortoises (Hagerty et al. in prep). Although we detected many potential barriers, these mountains and low elevation regions that can be characterized by extreme climatic conditions were most likely circumvented over hundreds of tortoise generations.

In these connectivity models, we identified important habitat corridors that would have acted as “pinch points.” For example, Las Vegas Valley was hypothesized previously to be a transitional corridor between populations in the northern and southern extent of the range (Britten et al. 1997). The probability that tortoises would use habitat in Las Vegas Valley, as well as the habitat on the east and west side of the Spring, New York, and Providence Mountains was extremely high (Hagerty et al. in prep). In contrast, habitat in California did not contain “pinch points” because topographic relief in that portion of the range is more homogenous.

Using genotype data, we gained a better understanding of historical ecological processes resulting in the visible population structure of the Mojave desert tortoise. These insights are valuable for implementing actions tailored to restore connectivity within the Mojave and Colorado Deserts. Although anthropogenic factors were not explicitly identified as shaping population structure (due to time lags in genetic signatures), we can generate specific hypotheses based upon inferences about habitat connectivity and current levels of habitat fragmentation. For example, major transportation infrastructure likely has severed connections among all desert tortoise subpopulations and habitat within

subpopulations in the Mojave and Colorado Deserts (Boarman et al. 1997, Edwards et al. 2004, Boarman and Sazaki 2006). Busy interstates and highways are a grave concern because they can be filters or complete barriers to movement (Clevenger and Wierzechowski 2006). Therefore, gene flow among tortoise subpopulations has been severely reduced, if not completely removed as an ecological process in this system.

Unfortunately, roads also can cause direct mortality of tortoises (Boarman et al. 1996, Boarman and Sazaki 2006), and fencing roads has been implemented to reduce the number of deaths (Boarman et al. 1996, Boarman et al. 1997). Although the reduction of mortality is positive, fencing further fragments habitat and reduces the likelihood of successful movement among subpopulations (Ruby et al. 1994, von Seckendorff Hoff and Marlow 2002, Edwards et al. 2004). Culverts under fenced roads may be one potential solution to reduce mortality of roads and allow connections among habitat (Clevenger and Wierzechowski 2006). Wildlife passages have been successful for other species, however, more hypothesis-driven research and monitoring is necessary to evaluate long-term effectiveness (Clevenger and Wierzechowski 2006). Although tortoises have successfully used culverts (Fusari 1985, Ruby et al. 1994, Boarman et al. 1996, Boarman et al. 1997), the effectiveness of culverts in facilitating population connectivity has not been addressed. Periodic translocation of individuals is an alternative management option for maintaining population connectivity. However, estimates of dispersal distances and rate of dispersal among subpopulations from intense field efforts will likely be required. The directionality and amount of dispersal necessary to maintain the genetic structure we identified is unclear (Frankham 2006).

## CONCLUSIONS

Despite the potential for conservation genetics to play a vital role in recovery planning for endangered species, few recovery plans request population genetic data and even less mention genetic factors as potential threats to population persistence (Moyle et al. 2003). Our goal was to describe how data from neutral genetic markers can be used in combination with other relevant data to make recommendations for a threatened species. As another method in the conservation biologist's toolbox, population genetic data are valuable to make important ecological inferences. In the case of the desert tortoise, we were able to identify boundaries for conservation units, propose a method to identify the population of origin for translocated individuals, and detect relevant corridors for movement among previously connected tortoise subpopulations. Genetic data should not be used in isolation to make recommendations for management actions, but genetic data and analyses can be extremely valuable when used with additional knowledge about the species of concern.

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## TABLES

Table 1. Assignment of resident individuals in the Large Scale Translocation Site near Jean, NV to one of nine genotype clusters identified using individual-based Bayesian assignment tests.

<b>Individual</b>	<b>Most likely population</b>	<b>Probability</b>
LS24	Amargosa	0.291
LS25	Not Assigned	-
LS26	South Las Vegas	0.705
LS27	South Las Vegas	0.298
LS28	Eldorado	0.088
LS29	South Las Vegas	0.526
LS30	South Las Vegas	0.346
LS31	South Las Vegas	0.078
LS32	Amargosa	0.076
LS33	South Las Vegas	0.516
LS34	South Las Vegas	0.094
LS35	South Las Vegas	0.039
LS36	South Las Vegas	0.136
LS37	South Las Vegas	0.172
LS38	South Las Vegas	0.166
LS39	South Las Vegas	0.459
LS40	South Las Vegas	0.21
LS41	South Las Vegas	0.057
LS42	South Las Vegas	0.118
LS43	South Las Vegas	0.377
LS44	South Las Vegas	0.174
LS45	South Las Vegas	0.186
LS46	Eldorado	0.132
LS47	South Las Vegas	0.117
LS48	Eldorado	0.290

Table 2. Assignment of translocated individuals in the Large Scale Translocation Site near Jean, NV to one of nine genotype clusters identified using individual-based Bayesian assignment tests.

<b>Individual</b>	<b>Most likely population</b>	<b>Probability</b>
LS01	South Las Vegas	0.073
LS02	Piute	0.130
LS03	Eldorado	0.073
LS04	South Las Vegas	0.524
LS05	Amargosa	0.151
LS06	South Las Vegas	0.205
LS07	South Las Vegas	0.137
LS08	Lower Virgin River	0.055
LS09	South Las Vegas	0.072
LS10	South Las Vegas	0.826
LS11	South Las Vegas	0.656
LS12	South Las Vegas	0.212
LS13	South Las Vegas	0.614
LS14	South Las Vegas	0.579
LS15	South Las Vegas	0.218
LS16	Eldorado	0.154
LS17	Eldorado	0.018
LS18	Amargosa	0.25
LS19	Not assigned	-
LS20	South Las Vegas	0.941
LS21	Not assigned	-
LS22	South Las Vegas	0.359
LS23	Muddy Mountains	0.29

Table 3. Private alleles found in nine subpopulations of the Mojave desert tortoise

<b>Sampling location</b>	<b>Subpopulation</b>	<b>Locus</b>	<b>Allele</b>	<b>Frequency</b>
BD	VR	GOA2	194	0.045
CS	MD	GOA13	206	0.019
CS	MD	GOA23	186	0.019
NEL	MD	GP15	280	0.025
SI	SLV	GOA14	303	0.018
SWL	SLV	GOA14	211	0.019
SWL	SLV	GOA17	299	0.018
SWL	SLV	GOA17	303	0.054
EL	EL	GOA22	143	0.010
PI	PI	GOA3	273	0.013
PI	PI	GOA4	263	0.044
PI	PI	GOA8	242	0.006
PI	PI	GOA14	283	0.007
PI	PI	GOA14	289	0.007
PI	PI	GOA17	241	0.007
PI	PI	GOA22	219	0.006
WP	NCO	GOA14	311	0.036
CM	NCO	GOA8	230	0.008
CM	NCO	GOA9	293	0.009
CM	NCO	GOA12	181	0.008
CM	NCO	GOA14	293	0.009
CM	NCO	GOA23	282	0.008
CM	NCO	GP15	222	0.009
EP	ECO	GOA11	341	0.014
EP	ECO	GP30	235	0.026
EP	ECO	GP61	251	0.013
CK	ECO	GOA2	191	0.009
CK	ECO	GOA8	238	0.009
CK	ECO	GOA12	118	0.018
CK	ECO	GOA22	131	0.009
CK	ECO	GOA23	182	0.009
PM	ECO	GP30	187	0.020
SC	WM	GOA6	284	0.011
SC	WM	GOA11	347	0.022
SC	WM	GOA22	127	0.011
FK	WM	GOA6	280	0.028
FK	WM	GOAG7	243	0.028

Table 4. Detection of 34 first generation migrants of the Mojave desert tortoise. Migrants detected between subpopulations where we did not have sufficient power to detect them are grayed, reducing the total number of migrants to 22.

	<b>Assigned cluster</b>								
<b>Home cluster</b>	VR	MD	AM	SLV	EL	PI	NCO	ECO	WM
VR	-	2		<b>2</b>	<b>2</b>				
MD	2	-							
AM		2	-	1	<b>1</b>	<b>1</b>			
SLV			1	-	1				
EL			<b>1</b>	1	-	<b>1</b>			
PI					<b>3</b>	-			
NCO			<b>1</b>	<b>3</b>	<b>1</b>	1	-		
ECO						<b>1</b>	<b>2</b>	-	<b>1</b>
WM							<b>1</b>	<b>2</b>	-

Table 5. Tests for population bottlenecks (BOTTLENECK and M-RATIO) in 25 sampling locations for the desert tortoise. The Wilcoxon significance test for population bottlenecks and the average M value and standard deviation are provided as well as results of the permutation test (percentage of runs in which expected M was lower than observed). Locations with less than 20 individuals are shown in gray. Only the South-I-15 corridor (SI, shown in red) had evidence for bottleneck using both tests.

Location	Wilcoxon Test (Bottleneck)	Average M (M- Ratio)	SD (M-Ratio)	Permutation test (M-Ratio)
RC	NS	0.712	0.211	0.05
BD	NS	0.657	0.288	0
MM	NS	0.738	0.225	0.28
GB	NS	0.626	0.265	0
MD	NS	0.749	0.202	0.43
CS	NS	0.735	0.230	0.25
NEL	NS	0.701	0.202	0.01
NWL	NS	0.714	0.189	0.08
AM	NS	0.685	0.213	0.01
PA	NS	0.718	0.182	0.10
SH	NS	0.657	0.206	0.01
IV	NS	0.637	0.227	0
WP	NS	0.663	0.227	0
SI	*	0.685	0.203	0.01
SWL	NS	0.753	0.189	0.48
SEL	NS	0.623	0.241	0
EL	NS	0.755	0.178	0.6
PI	NS	0.808	0.149	5.71
CM	NS	0.777	0.161	1.46
EP	NS	0.756	0.195	0.7
CK	NS	0.747	0.188	0.32
PM	NS	0.720	0.220	0.14
OR	NS	0.649	0.228	0.01
SC	NS	0.695	0.173	0.01
FK	NS	0.635	0.233	0

## FIGURE LEGENDS

Figure 1. Depiction of recovery units delineated in the 1994 Recovery Plan for the Mojave population of the desert tortoise: Upper Virgin River (dark purple), Northeastern Mojave (green), Eastern Mojave (brown), Northern Colorado (grey), Eastern Colorado (light purple), and Western Mojave (pink). Green lines within the recovery units are proposed Desert Wildlife Management Areas, gray lines are state boundaries, and black lines indicate interstate freeways (recreated from Tracy et al. 2004).

Figure 2. Map of the listed geographic range of the Mojave population of the desert tortoise. Each point indicates each location where a blood sample was collected. The color of the marker further indicates the subpopulation (Virgin River = red, Muddy Mountains = light blue, Amargosa Desert = orange, South Las Vegas = dark blue, Eldorado Valley = teal, Piute Valley = purple, Northern Colorado = green, Eastern Colorado = yellow, Western Mojave = pink). Black lines indicated interstate highways. Figure is reprinted from Hagerty and Tracy in prep.

Figure 3. Proposed revisions to conservation units for the Mojave desert tortoise. The color of the marker further indicates each unit: Upper Virgin River = purple, Lower Virgin River = red, Muddy Mountains = light blue, Amargosa Desert = orange, South Las Vegas = dark blue, Northern Colorado = green, Eastern Colorado = yellow, Western Mojave = pink. Some shaded areas overlap due to lack of clarity for where boundaries occur. Green lines indicate Desert Wildlife Management areas, which are areas of active management for the recovery of the Mojave desert tortoise.

**FIGURES**

Figure 1.

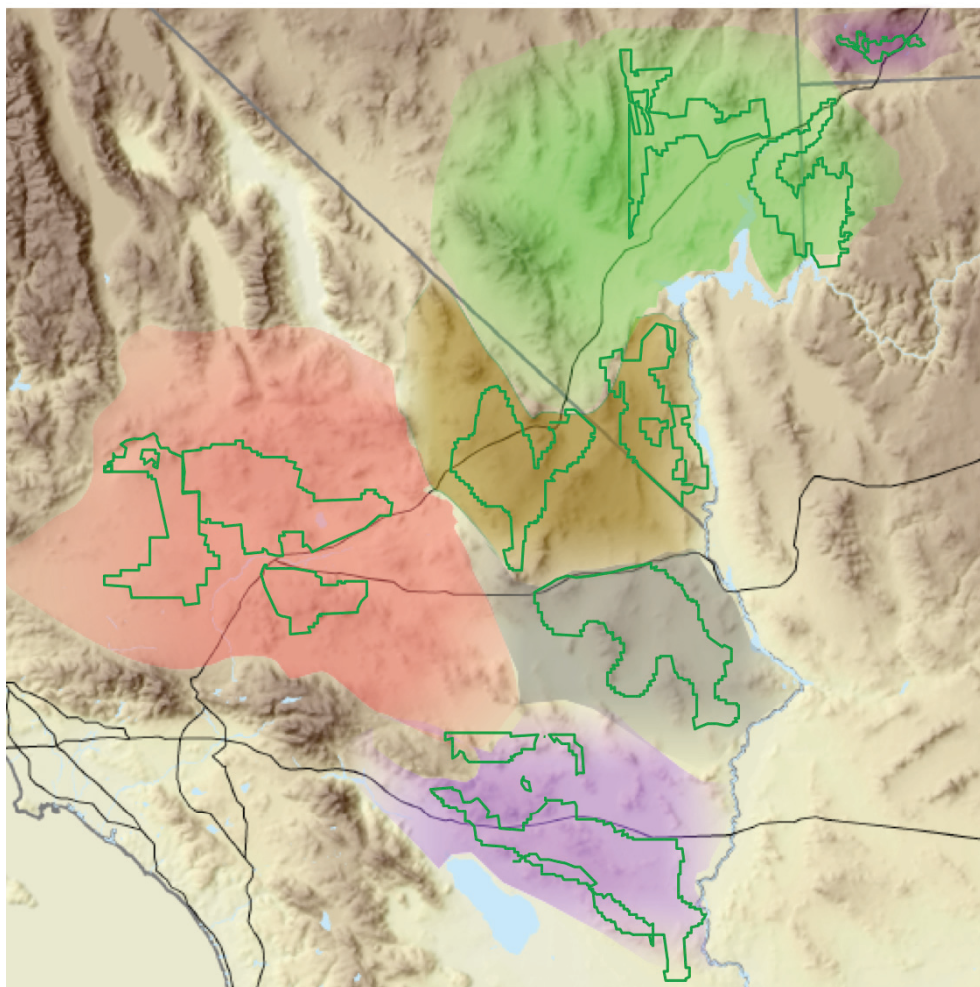


Figure 2.

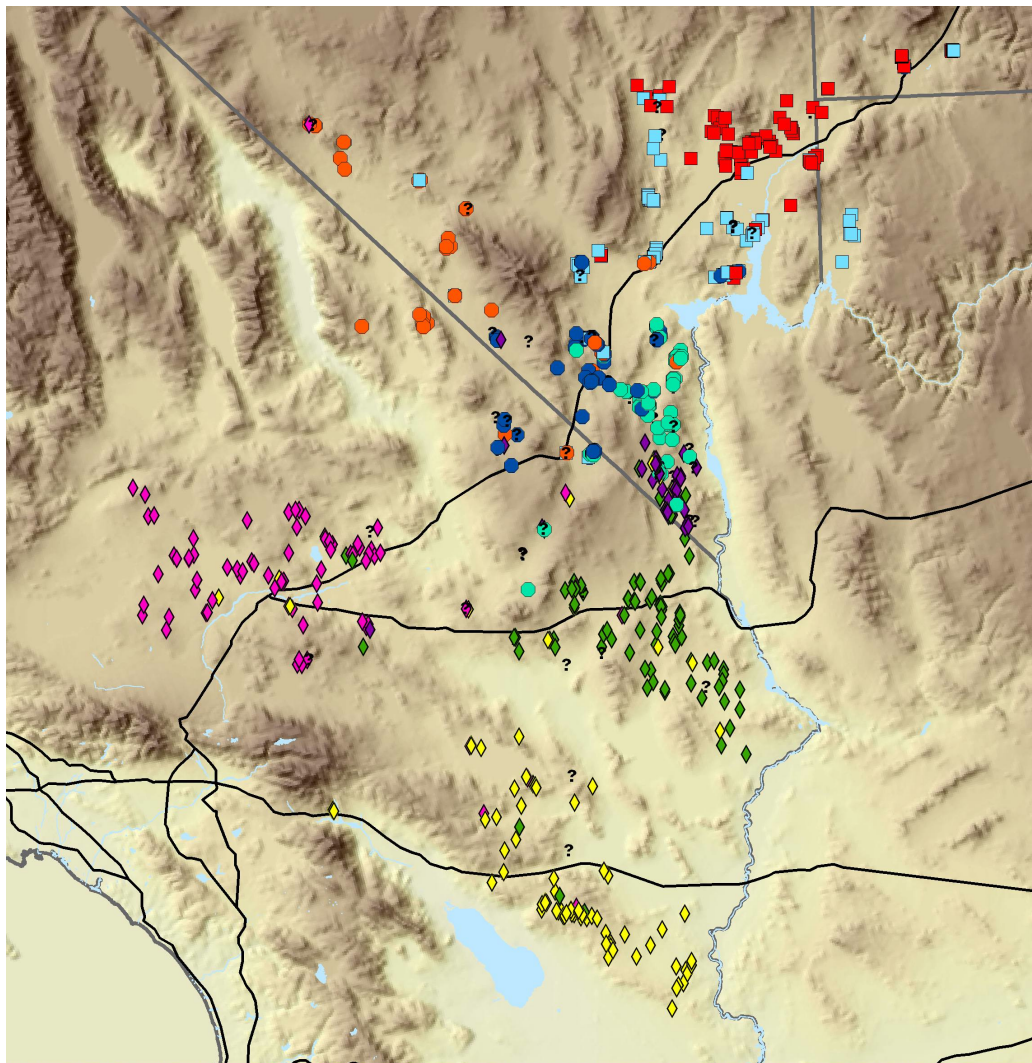
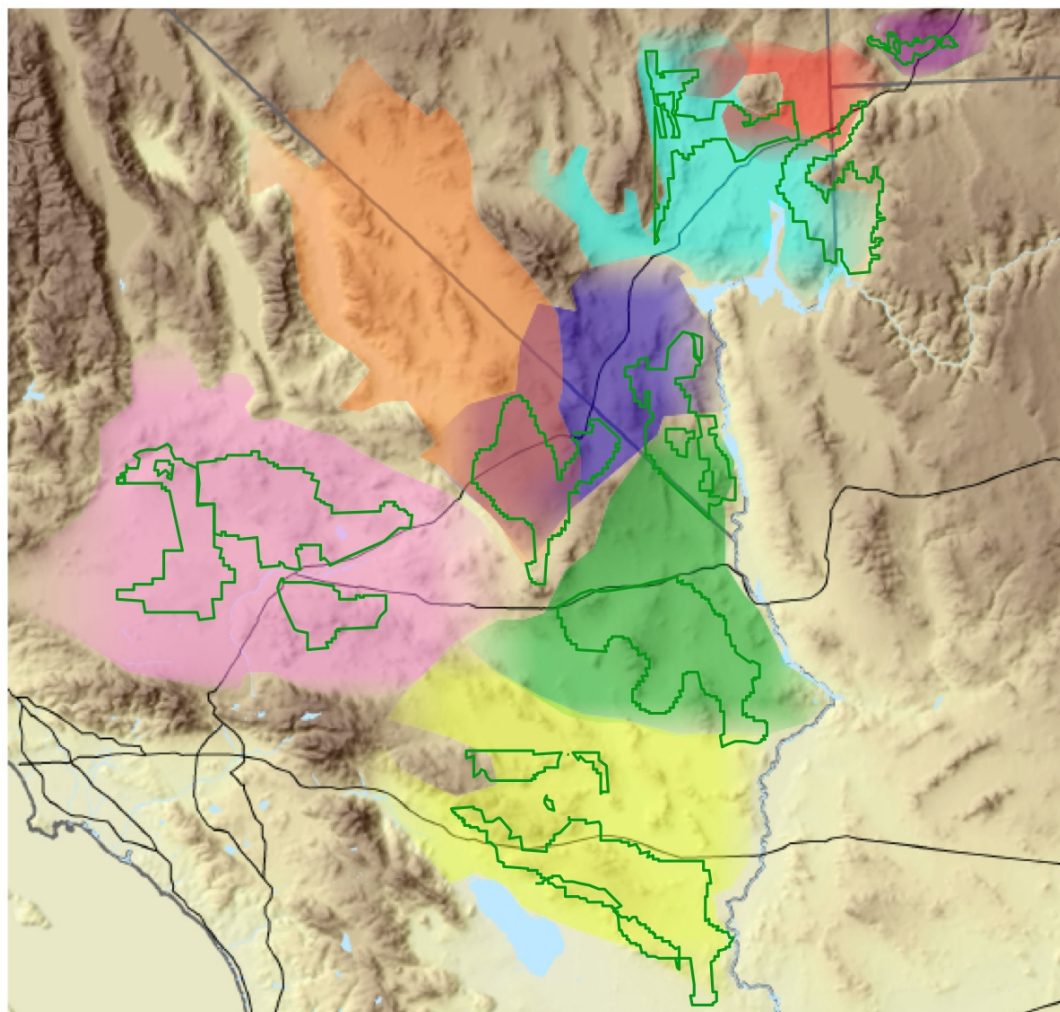


Figure 3.



**APPENDIX A. PRIMER NOTE FOR MICROSATELLITE MARKERS FOR THE  
DESERT TORTOISE**

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**Polymorphic microsatellite markers for the Mojave desert tortoise, *Gopherus agassizii***

Bridgette E. Hagerty<sup>1,2</sup>, Mary M. Peacock<sup>2</sup>, Veronica S. Kirchoff<sup>2</sup>, and C. Richard Tracy<sup>2</sup>

<sup>1</sup>Program in Ecology, Evolution, and Conservation Biology  
University of Nevada, Reno  
Reno, NV 89557  
USA

<sup>2</sup>Department of Biology  
University of Nevada, Reno  
Reno, NV 89557  
USA

**Corresponding Author:** Bridgette E. Hagerty  
Department of Biology, MS/314  
University of Nevada, Reno  
Reno, NV 89557  
USA  
Fax: (775) 784-1369  
[bh@biodiversity.unr.edu](mailto:bh@biodiversity.unr.edu)

**Keywords:** *Gopherus agassizii*, desert tortoise, microsatellites, PCR primers, Mojave  
Desert, conservation genetics

## Abstract

We describe primers and polymerase chain reaction (PCR) conditions to amplify 14 tri- and tetra-nucleotide microsatellite loci for the Mojave desert tortoise (*Gopherus agassizii*). Across three populations (87 individuals) located in the Mojave Desert, USA, the markers yielded a range of 4 to 33 alleles and an average observed heterozygosity of 0.733 (range 0.433 –0.933). We did not detect linkage disequilibrium between any pair of loci, nor did we find a consistent pattern of deviation from Hardy-Weinberg equilibrium. These microsatellites are designed for PCR multiplexing, and provide higher throughput capacity to aid in conservation genetics studies for this threatened species.

## Primer Note

The desert tortoise (*Gopherus agassizii*) is federally listed as threatened under the United States Endangered Species Act (1973) in the part of its range that occurs north and west of the Colorado River (USFWS 1994). The range of the Mojave desert tortoise spans four states and multiple habitat types across the Mojave and Colorado Deserts. Habitat destruction and fragmentation, invasive plants, and other threats related to increased human access to the desert have caused serious population declines (USFWS 1994). Therefore, determining genetic population structure and rates of gene flow among populations is extremely important for developing conservation and management actions. We describe 14 novel microsatellite loci for the Mojave population of the desert tortoise (Table 1). These markers can be used in conjunction with markers previously developed

for the Sonoran population of the desert tortoise (Edwards et al. 2004) and the gopher tortoise (*Gopherus polyphemus*; Schwartz et al. 2003) to address population-level questions important to the conservation of this distinct population segment.

Markers were isolated from four tri- and tetra- nucleotide microsatellite-enriched genomic libraries produced by Genetic Identification Services (GIS) (<http://www.genetic-id-services.com/>). In the (ATG) library, 11 of 24 clones contained a microsatellite, 3 of 9 clones in the (CAG) library contained a microsatellite, 13 of 24 clones contained a microsatellite in the (CATC) library, and 32 of 39 clones contained a microsatellite in the (TAGA) library. GIS developed primers from cloned sequences for 32 loci using DesignerPCR, version 1.03 (Research Genetics, Inc.), and synthesized and tested 24 of those loci. Fluorescently-labeled primers for these 24 loci were ordered from Applied Biosystems (ned, pet, vic) and Operon (fam) at the University of Nevada, Reno and were screened for polymorphism. Monomorphic or inconsistent loci across 8 -20 individuals were not pursued; we screened the remaining 14 loci with additional individuals.

Whole blood was collected from desert tortoises and dried onto filter paper. Total genomic DNA was extracted from filter paper dots using a dried blood protocol for QIAGEN DNeasy kits (Qiagen 2001). DNA was eluted in a TE buffer, quantified using a Labsystems Fluoroskan Ascent fluorometer, and diluted to total genomic concentrations between 15 -20 ng.

Microsatellites were amplified in one of four multiplex polymerase chain reactions (PCR) or as a single PCR (Table 1). All multiplex reactions contained 1x Multiplex PCR Master Mix (QIAGEN), 0.2  $\mu$ M multiplex primer cocktail, and 60-80 ng genomic DNA in a 16 $\mu$ L PCR reaction. Individual primer concentrations were adjusted in the multiplex cocktail to produce equal intensities for fragment analysis. All multiplex PCR cycling was performed using a MBS Satellite 0.2G thermal cycler with the following profile: 1 cycle of 94°C for 15 min, 33 cycles of 94°C for 30s, appropriate annealing temperature ( $T_a$ ) for 90s (Table 1), 72°C for 30s, and 1 cycle of 62°C for 30min. Multiplex 1 contained primers GOA2, GOA8, and GOA13 ( $T_a$  = 59°C). Multiplex 2 contained the primers GOA1, GOA6, GOA11, and GOA12 ( $T_a$  = 61°C). Multiplex 3 contained the primers GOA4, GOA22, and GOA23 ( $T_a$  = 61°C). Multiplex 4 contained the primers GOA3, GOA9, and GOA14 ( $T_a$  = 61°C).

GOA17 was amplified in single PCR reactions using a MBS Satellite 0.2G thermal cycler. The 15- $\mu$ L reactions contained 1x Titanium taq PCR buffer (pH 8.0, 3.5mM MgCl<sub>2</sub>) (CLONTECH Laboratories, Inc.), 0.2 units Titanium taq DNA polymerase (CLONTECH Laboratories, Inc.), 0.25 mM dNTPs, 0.2 $\mu$ M forward and reverse primer, and 60-80 ng genomic DNA. Cycling conditions were 1 cycle of 94°C for 1min, 33 cycles of 94°C for 30s, 61°C for 30s, 72°C for 30s, and 1 cycle of 72°C for 30min.

All amplified products underwent a multi-color fluorescence-based DNA fragment size analysis in four separate panels using an ABI 3730 DNA Analyzer, and

alleles were scored with GENEMAPPER 3.7 software (Applied Biosystems). After optimization of PCR conditions, microsatellite amplification and fragment analysis were completed at the Nevada Genomics Center (<http://www.ag.unr.edu/Genomics/>).

Polymorphism was assessed at these loci using 87 individuals from three disparate populations of *Gopherus agassizii* in the Mojave Desert, USA. Observed and expected heterozygosity, and the number of alleles per locus were calculated using GENEPOP (Raymond and Rousset 1995) (Table 2). All microsatellites were highly polymorphic, with observed allele numbers ranging from 4 to 33 across all populations (Table 1). Tests for deviation from genotypic equilibrium and Hardy-Weinberg equilibrium were performed using FSTAT (version 2.9.3.2; Goudet 2001). No pair of loci exhibited linkage disequilibrium in any group after Bonferroni correction ( $P < 0.00006$ ), and pairs of loci that were significantly linked prior to Bonferroni correction ( $P < 0.05$ ) showed no consistent pattern.  $F_{IS}$  values were used to quantify any deviation from Hardy-Weinberg equilibrium. Three significant heterozygote deficits were identified after Bonferroni correction ( $P < 0.0004$ ). GOA6 had a significant heterozygote deficit in the Utah population; GOA22 had a significant deficit in the Nevada population, and GOA9 had a significant deficit in the California population (Table 2). However, no sampled population (global test) had a significant deviation from Hardy Weinberg equilibrium after the correction and  $F_{IS}$  values showed no consistent pattern within populations among loci or across populations per locus (Table 2). The combined probability of expected heterozygote classes ( $P < 0.05$ ) in MICRO-CHECKER (version 2.2.3; van Oosterhout et al. 2004) supported these significant  $F_{IS}$  values and suggested the presence of null alleles.

The evidence for the presence of null alleles also was not consistent among sampled populations; therefore, we concluded that each locus was suitable for future analyses.

### **Acknowledgements**

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Table 1. Desert tortoise microsatellite loci (*Gopherus agassizii* (GOA)) combined in 4 multiplex PCR primer sets and one single PCR. Forward (F) and reverse (R) primer sequences, product size range (bp), total number of individuals (N), number of alleles per locus (A), the PCR annealing temperature ( $T_a$ ), and the GenBank Accession number are provided for each locus.

Multiplex Set	Locus name - dye	Repeat in clone	Primer (5'-3')	Product size range (bp)	$T_a$ (°C)	N	A	Genbank Accession no.
NA	GOA17-ned	(CTAT) <sub>16</sub>	F: ATATGCCCCCTGGTATGAG R: TACTGGGACATGAAGGAAGTG	203-295	61	87	21	EU285466
Set 1	GOA2-fam	(GCT) <sub>6</sub> (GAGCACTA GGACCTC) (GCT) <sub>5</sub> (CTTCGT) (GCT) <sub>2</sub>	F: TTCTAGCAGGCTCCTGATTAC R: AGGGATGGAAGTGGTAGTCTC	200-206	59	87	4	EU285462
	GOA8-pet	(TAGA) <sub>16</sub>	F: TGGTAACAGAATCCAGGAGTTC R: GGAGCGAGGCTTAGGAGAC	162-194	59	87	16	EU285470
	GOA13-vic	(CTAT) <sub>11</sub>	F: ACCCTAAAGCGTGAAAGTATC R: AAGTAGTCCCCACAGAGTGAC	205-253	59	87	15	EU285465
Set 2	GOA1-fam	(CATC) <sub>7</sub>	F: GGACTCCAGACCTGTATGG R: GCAGCCTTTGAAAACTTG	123-135	61	87	4	EU285463
	GOA6-ned	(CTAT) <sub>10</sub>	F: GCAGTGGAATAACAGTAGGAC R: AGGATGGATGGATTAGATGAC	204-264	61	87	18	EU285464
	GOA11-pet	(CAT) <sub>8</sub>	F: TACGGTATCCCCGACGAG R: CTGGCACAATGGTCCTTG	263-327	61	87	21	EU285458
	GOA12-vic	(CAT) <sub>37</sub>	F: ATGGTGCTACGAACACATTC R: TTTCTCTTCTGCGAACAC	99-158	61	87	16	EU285461
Set 3	GOA4-fam	(CAT) <sub>11</sub>	F: GCCTCTGTCACTTATGTTTCATG R: AAGAAACCTCTCCAAGTACGTC	257-281	61	87	13	EU285460
	GOA22-vic	(CTAT) <sub>18</sub>	F: AGTGCCTACTCAGTTTCTACCC R: GGGATTGATGCCAGTTCTAC	147-215	61	87	19	EU285467
	GOA23-pet	(TAGA) <sub>18</sub>	F: GGAGGGTGCTAAGATACTCTG R: TTCAGGTGTGTTTCCACATC	190-258	61	87	18	EU285469
Set 4	GOA3-fam	(CAT) <sub>7</sub>	F: ACATTTACAGGGGAGACTCAG R: ATCTGTGGGAGAAGAATATCTG	275-282	61	87	5	EU285459
	GOA9-pet	(CATC) <sub>19</sub> (CCAT) (CTAT) <sub>8</sub>	F: TCTTGGCATTACAAACATC R: TCCCACTGGAACTTCATTC	205-289	61	87	18	EU285468
	GOA14-vic	(TAGA) <sub>24</sub>	F: AGGTGCTCAGATACCACAGTC R: CCCATCGAATCTCTTTAATG	218-251	61	87	33	EU285471

Table 2. Summary per locus across three populations of the desert tortoise in the Mojave Desert, USA. Number of individuals sampled (N), number of alleles found in the population (A), allelic richness ( $R_s$ ), observed and expected heterozygosity ( $H_o / H_e$ ), and  $F_{IS}$  values are reported. Significant heterozygote deficits after Bonferroni correction ( $P < 0.0004$ ) are in bold font.

Locus name	Red Cliffs Desert Reserve, UT					Pahrump Valley, NV					Superior-Cronese Desert Wildlife Management Area, CA				
	N	A	$R_s$	$H_o / H_e$	$F_{IS}$	N	A	$R_s$	$H_o / H_e$	$F_{IS}$	N	A	$R_s$	$H_o / H_e$	$F_{IS}$
GOA17	30	16	14.9	0.93 / 0.88	-0.061	27	15	14.7	0.89 / 0.92	0.041	30	11	10.4	0.87 / 0.85	-0.021
GOA2	30	3	2.8	0.23 / 0.21	-0.097	27	3	2.0	0.15 / 0.18	0.158	30	3	3.0	0.43 / 0.50	0.141
GOA8	30	11	10.7	0.90 / 0.86	-0.043	27	11	10.9	0.89 / 0.85	-0.050	30	12	11.6	0.87 / 0.86	-0.004
GOA13	30	10	9.3	0.83 / 0.76	-0.098	27	10	9.7	0.59 / 0.77	0.235	30	9	8.5	0.53 / 0.55	0.023
GOA1	30	3	3.0	0.57 / 0.49	-0.165	27	4	4.0	0.70 / 0.65	-0.086	30	2	2.0	0.63 / 0.50	-0.264
GOA6	30	11	10.6	0.57 / 0.89	<b>0.367</b>	27	11	11.0	0.85 / 0.89	0.045	30	14	12.7	0.83 / 0.85	0.018
GOA11	30	11	10.1	0.47 / 0.57	0.186	27	12	11.5	0.82 / 0.81	-0.005	30	12	11.5	0.87 / 0.85	-0.022
GOA12	30	6	5.6	0.50 / 0.53	0.055	27	9	8.7	0.93 / 0.82	-0.136	30	14	13.1	0.83 / 0.88	0.049
GOA4	30	10	9.3	0.53 / 0.69	0.226	27	9	8.7	0.67 / 0.74	0.097	30	6	12.7	0.67 / 0.70	0.054
GOA22	30	10	9.6	0.63 / 0.83	0.244	27	12	11.8	0.56 / 0.89	<b>0.381</b>	30	17	15.9	0.97 / 0.92	-0.055
GOA23	30	11	10.1	0.93 / 0.80	-0.173	27	16	15.4	0.93 / 0.91	-0.013	30	12	11.4	0.93 / 0.91	-0.030
GOA3	30	4	4.0	0.70 / 0.72	0.026	27	5	4.9	0.74 / 0.72	-0.026	30	3	3.0	0.37 / 0.34	-0.078
GOA9	30	15	14.1	0.80 / 0.91	0.117	27	13	12.7	0.74 / 0.91	0.190	30	12	12.5	0.55 / 0.90	<b>0.389</b>
GOA14	30	19	17.4	0.93 / 0.91	-0.024	27	21	20.4	0.92 / 0.95	0.024	30	18	17.2	0.87 / 0.93	0.073

## APPENDIX B: SUPPLEMENTAL METHODS AND RESULTS FOR CHAPTER 1

### Supplemental Description of Population Genetic Analyses

#### *Bayesian Clustering:*

**STRUCTURE** (Pritchard et al. 2000, Falush et al. 2003, Pritchard et al. 2007):

*STRUCTURE* uses Bayesian clustering to identify a set of inferred genetic populations ( $K$ ) and to probabilistically assign individuals to those populations based on their genotypes.

In the *STRUCTURE* model, there are a true underlying number of genotype clusters ( $K$ ), which may or may not be known. The model assumes that the genetic markers are unlinked (not in linkage disequilibrium) and in Hardy-Weinberg equilibrium within each of the genotype clusters. Individuals probabilistically are assigned to one (no admixture) or more than one (admixture) of the inferred genotype clusters, which are characterized by allele frequency distributions that satisfy the assumptions of the model. Admixture simply allows for the genetic makeup of an individual to be derived from more than one inferred genotype cluster. Using the known genotypes of sampled individuals ( $X$ ), the unknown parameters (the population of origin, ( $Z$ ), allele frequencies of each population ( $P$ ), and individual's proportion of admixture ( $Q$ ) are estimated using Markov Chain Monte Carlo (MCMC) re-sampling algorithms. When using the admixture model, the vector  $Z$  pertains to each allele copy, not each individual, to allow for a multiple allele origins within each individual.

For a particular data set, individuals can be grouped into  $K$  clusters. Each allele from an individual's genotype is treated as a random draw from the appropriate cluster (s)'s allele frequency distribution. These random draws of alleles from the frequency distribution  $P$  for an unknown population  $Z$  describe the probability distribution  $Pr(X | Z, P, Q)$ . To infer the unknown parameters of interest, a Bayesian approach is used and priors for  $Pr(Z)$  and  $Pr(P)$  are specified. If no prior information is included about the population of origin, the probability that an individual originated from a population ( $k$ ) is the same for all  $k$ . A Dirichelet distribution is used to model allele frequencies at each  $k$  because this distribution has the property that the frequencies sum to 1. This distribution is also used to model admixture proportions ( $q$ ), and a uniform prior is used to learn about the level of admixture from the data.

The posterior distribution to determine  $Z$  and  $P$  was:  $Pr(Z, P, Q | X) \propto Pr(Z) Pr(P) Pr(Q) Pr(X | Z, P)$ . Obtaining a posterior distribution in Bayesian analyses, such as in STRUCTURE, is computationally difficult. Therefore, draws from the priors were used to approximate the posterior distribution using a Markov Chain Monte Carlo (MCMC) re-sampling algorithm. The goal of the algorithm is to construct Markov chains of the parameters with a sufficiently large burn-in ( $m$ ) and thinning interval ( $c$ ), resulting in a stationary distribution of the posterior distribution  $Pr(Z, P, Q | X)$ , where samples from the distribution are independent of the starting values. The Gibbs sampling method was used to construct the Markov chains.

Obtaining a posterior distribution in Bayesian analyses, such as in STRUCTURE, is computationally difficult and often requires the integration of high-dimensional functions. As a result, many Bayesian models use other analytical methods to achieve a

posterior distribution. MCMC methods attempt to simulate direct draws from a complex distribution of interest (in our case, we simulated draws from the priors to approximate the posterior distribution). A Markov chain uses the previous sample to randomly generate the next sample value (transitional probabilities are the probability that a state space (potential range of values of  $X$ ) changes from one state to another in a single step and are only a function of the current sample value). Over time steps as the chain evolves it eventually is no longer dependent on the starting value, which means that the chains has reached a stationary distribution. A burn-in period is needed to obtain a stationary distribution. The Monte Carlo approach uses random number generation to approximate posterior distributions. In order to apply the Monte Carlo approach, we need to obtain values from a complex probability distribution. One way to solve this problem is the Metropolis- Hastings algorithm. Gibbs sampling is a special case of this algorithm, and is used in STRUCTURE.

In general, determining the size of the burn-in and thinning interval is not obvious. The value of  $m$  was determined by evaluating whether the inferred values of the parameters (*e.g.*,  $\ln Pr(X/K)$ ) from the posterior distributions were similar across independent runs of the model. We chose 750,000 iterations for  $m$ , and used 750,000 iterations of the chain to approximate the posterior distributions. STRUCTURE determined the natural log of the probability of the data given a certain number of clusters ( $\ln Pr(X/K)$ ) for each value of  $K$ .

The more likely number of clusters is inferred from the estimated log probability of data  $Pr(X / K)$  for each  $K$  (Pritchard et al. 2000, Pritchard et al. 2007). After each step of the MCMC re-sampling algorithm, the program computes the log-

likelihood of the data (Pritchard et al. 2000). For each independent simulation, the mean of the log-likelihood values is computed and half their variance is subtracted from the mean. The resulting output value is known as the “estimated natural log of the probability of data” or  $\ln P(D)$  (see equation 12 in Pritchard et al. 2000). To infer the most likely number of genotype clusters, we calculated the mean  $\ln P(D)$  across 10 independent simulations for  $K=1$  through  $K=10$ . We chose the value of  $K$  that maximized the mean log-likelihood. This estimation of the most probable  $K$  should only be considered a guide. Typically, the highest  $\ln P(D)$  prior to a plateau is used as a rule of thumb for choosing the most likely  $K$ . In essence, the smallest value of  $K$  that explains the most structure in the data should be used as the most parsimonious solution.

Calculating  $\Delta K$  provides a more formal criterion for determining the most probable number of genotype clusters (Evanno et al. 2005). Because the values of  $\ln P(D)$  often plateau at larger  $K$ s, the amount of change in the log likelihood values is larger at lower values of  $K$  and decreases as  $K$  increases. As a result, the second order rate of change of the likelihood function with respect to  $K$  ( $\Delta K$ ) was identified as a valuable tool in estimating the true number of genotype clusters. Three steps are necessary to calculate  $\Delta K$  (using the notation of Evanno et al. 2005,  $\ln P(D)$  is referred to as  $L(K)$ ) :

1. Calculate the mean likelihood ( $\ln P(D)$ ) across multiple independent simulations for each value of  $K$  (i.e. mean  $L(K)$  )
2. Plot the mean difference for successive likelihood values of  $K$  ( $L'(K)$ ), where  $L'(K) = L(K) - L(K-1)$
3. Plot the absolute value of the difference between successive values ( $L''(K)$ ), where  $|L''(K)| = |L'(K+1) - L'(K)|$
4.  $\Delta K = m(|L''(K)|) / s[L(K)]$  , where  $m$  is the mean and  $s$  is the standard deviation of the independent simulations

## Supplemental Tables and Figures from Chapter 1

### Supplemental Tables

Table 1. Mean proportional membership of each desert tortoise sampling location to nine genotype clusters as identified by STRUCTURE.

Population	Mean proportional membership in genotype cluster (1-9)								
	1	2	3	4	5	6	7	8	9
RC	0.64	0.14	0.02	0.07	0.03	0.04	0.02	0.02	0.02
BD	0.88	0.05	0.04	0.01	0.01	0.01	0.00	0.00	0.01
MM	0.88	0.05	0.01	0.01	0.01	0.01	0.00	0.00	0.00
GB	0.48	0.46	0.01	0.01	0.01	0.01	0.00	0.01	0.01
MD	0.15	0.60	0.02	0.14	0.04	0.01	0.01	0.01	0.01
CS	0.29	0.50	0.08	0.05	0.03	0.03	0.01	0.01	0.01
NEL	0.06	0.68	0.09	0.10	0.03	0.02	0.01	0.01	0.01
NWL	0.06	0.43	0.12	0.26	0.08	0.02	0.01	0.01	0.01
AM	0.04	0.08	0.69	0.03	0.05	0.02	0.01	0.02	0.05
PA	0.02	0.06	0.66	0.14	0.05	0.04	0.01	0.01	0.01
SH	0.02	0.07	0.42	0.26	0.08	0.07	0.03	0.02	0.03
IV	0.07	0.09	0.15	0.30	0.21	0.11	0.02	0.02	0.02
WP	0.02	0.07	0.09	0.14	0.19	0.22	0.07	0.09	0.12
SI	0.04	0.12	0.11	0.50	0.17	0.03	0.01	0.02	0.01
SWL	0.05	0.11	0.09	0.56	0.13	0.03	0.01	0.01	0.02
SEL	0.04	0.07	0.17	0.31	0.28	0.08	0.02	0.02	0.02
EL	0.02	0.03	0.04	0.16	0.60	0.07	0.03	0.02	0.02
PI	0.01	0.02	0.02	0.04	0.11	0.45	0.21	0.09	0.05
CM	0.00	0.01	0.01	0.01	0.01	0.03	0.70	0.17	0.06
EP	0.01	0.01	0.01	0.01	0.02	0.06	0.60	0.16	0.12
CK	0.01	0.01	0.01	0.01	0.01	0.02	0.15	0.75	0.04
PM	0.01	0.01	0.01	0.01	0.01	0.04	0.18	0.49	0.23
OR	0.00	0.01	0.01	0.01	0.01	0.06	0.12	0.09	0.69
SC	0.01	0.01	0.01	0.01	0.01	0.02	0.07	0.04	0.82
FK	0.01	0.01	0.01	0.01	0.01	0.02	0.03	0.06	0.86

Table 2. Analysis of molecular variance for 9 genotype clusters as determined via STRUCTURE. \* significance at  $p < 0.05$  as determined by 1023 permutations

<b>Source of Variation</b>	<b>df</b>	<b>Sum of Squares</b>	<b>Variance components</b>	<b>Percentage variation</b>
Among groups	8	632.49	0.367	4.72*
Among populations within groups	16	230.34	0.138	1.78*
Among individuals within populations	723	5468.89	0.300	3.87*
Within populations	748	5208.5	6.96	89.64*
Total	1495	11540.22	7.77	

Table 3. Analysis of molecular variance for 4 genotype clusters as determined via GENELAND. \* significance at  $p < 0.05$  as determined by 1023 permutations

<b>Source of Variation</b>	<b>df</b>	<b>Sum of Squares</b>	<b>Variance components</b>	<b>Percentage variation</b>
Among groups	3	469.56	0.378	4.83*
Among populations within groups	21	393.27	0.195	2.49*
Among individuals within populations	723	5468.89	0.300	3.83*
Within populations	748	5208.5	6.96	88.85*
Total	1495	11540.22	7.84	

Table 4.  $F_{IS}$  values for 25 sampling location of the desert tortoise across 20 microsatellite loci. Bold values indicate significance after the Bonferroni correction, and red values indicate significance prior to the alpha correction.

	RC	BD	MM	GB	MD	CS	NEL	NWL	AM	PA	SH	IV	WP	SI	SWL	SEL	EL	PI	CM	EP	CK	PM	OR	SC	FK
GOA1	-0.16	NA	0.19	0	0.15	0.06	-0.15	0.06	-0.14	-0.09	-0.12	-0.49	0.06	-0.08	0.06	0.06	-0.11	0.1	0.15	0.33	0.05	0.01	-0.11	-0.36	0.23
GOA2	-0.09	-0.02	-0.02	NA	0	0	NA	0.15	0.46	0.16	0.26	-0.04	0.05	-0.11	-0.13	-0.2	0.04	-0.07	0.06	0.18	0.09	-0.08	-0.16	0.01	0.42
GOA3	0.03	0.06	-0.03	0.46	-0.13	-0.04	0.27	0.11	-0.17	-0.03	0.07	0.06	0.04	-0.13	0.08	-0.06	-0.03	0.05	0.10	-0.04	-0.13	-0.09	-0.18	0.04	0.42
GOA4	0.22	-0.21	-0.1	-0.11	-0.03	0.06	-0.12	-0.13	-0.07	0.1	-0.1	-0.11	-0.1	0.08	0.12	-0.19	0.06	0.02	0.09	0.04	-0.12	-0.05	0.01	-0.01	-0.08
GOA6	<b>0.32</b>	0.33	0.15	<b>0.64</b>	<b>0.53</b>	0.19	0.09	0.16	0.42	0.05	0.32	0.30	0.15	0.1	0.09	0.44	<b>0.33</b>	<b>0.24</b>	0.02	0.07	-0.02	0.15	0.16	0.00	-0.04
GOA8	-0.05	0.12	-0.02	-0.05	0.01	0.04	-0.04	0.12	0.02	-0.05	-0.14	0.1	-0.11	-0.07	0.02	-0.02	0.05	0.03	-0.05	-0.04	0.00	0.03	0.09	-0.03	0.05
GOA9	0.10	0.46	0.12	-0.02	0.06	0.25	-0.03	-0.02	0.17	0.19	0.15	0.22	0.09	0.21	0.19	0.01	0.00	0.07	0.12	0.14	0.05	0.18	<b>0.55</b>	<b>0.41</b>	0.11
GOA11	0.13	0.18	0.1	0.04	0.16	0.01	0.08	0.21	0.1	-0.01	0.17	0.03	0.07	-0.01	-0.06	0.02	0.12	<b>0.19</b>	0.27	0.00	-0.03	0.01	0.13	-0.01	0.20
GOA12	0.03	-0.1	-0.14	0.22	0.06	0.12	0.05	0.17	0.08	-0.14	0.23	-0.1	0.31	-0.02	-0.03	0.24	0.08	0.11	0.18	0.22	<b>0.59</b>	0.32	0.28	0.13	0.23
GOA13	-0.1	-0.07	0.07	0.26	0.11	-0.00	0	-0.00	-0.04	0.24	0.02	-0.07	0.04	-0.04	-0.26	-0.08	0.06	-0.07	0.07	0.10	0.02	0.12	-0.15	-0.04	-0.13
GOA14	0.01	-0.14	-0.03	-0.03	0.02	0.03	-0.04	0.00	0.01	0.02	0.08	0.15	0.03	-0.06	0.01	0.12	0.06	0.08	0.06	-0.08	-0.01	0.07	0.02	0.05	0.07
GOA17	-0.08	-0.11	-0.12	0.04	0.05	-0.00	-0.02	-0.03	0.01	0.04	0.05	0.09	-0.05	0.09	-0.07	-0.08	0.05	-0.08	0.00	0.02	-0.03	0.02	-0.11	-0.02	0.03
GOA22	0.21	0.08	0.22	0.08	0.06	0.12	-0.03	-0.08	0.09	<b>0.38</b>	0.02	0.21	0.01	-0.01	0.09	-0.03	0.01	-0.01	-0.00	0.1	0.02	0.05	0.12	-0.01	0.03
GOA23	-0.13	0.14	-0.08	-0.02	-0.06	0.01	-0.08	-0.01	-0.04	-0.01	0.09	-0.03	-0.1	-0.05	0.07	0.18	-0.01	-0.02	0.04	0.01	-0.02	-0.01	-0.07	-0.01	-0.15
GOAG3	0.54	0.15	-0.12	-0.10	-0.12	-0.15	0	-0.03	-0.13	0.52	-0.2	0	-0.04	0.01	-0.13	0.43	-0.01	-0.03	NA	0	NA	NA	NA	NA	NA
GOAG4	0.06	0.41	0.02	0.20	0.01	0.02	-0.09	0.1	0.03	-0.09	-0.03	0.05	0.09	0.05	-0.04	-0.03	0.05	0.07	-0.06	-0.04	-0.01	0.08	-0.01	0.01	0.03
GOAG7	-0.06	0.04	-0.17	0.40	-0.04	-0.09	-0.22	0.06	-0.03	0.18	-0.08	-0.09	-0.01	-0.11	-0.13	0.02	0.07	0.05	0.02	-0.02	0.03	-0.01	-0.07	-0.20	-0.02
GP15	0.18	0.02	-0.02	0.11	0.19	0.12	0.03	-0.00	-0.03	-0.01	-0.01	-0.01	0.09	0.01	0.03	0	-0.11	0.02	0.08	-0.07	-0.11	0.06	-0.16	0.07	0.05
GP30	0.11	-0.42	0	0.01	0.05	0.06	0.00	0.29	-0.01	-0.01	-0.15	0.05	-0.03	-0.04	0.10	0.04	-0.06	-0.06	-0.03	0.16	0.15	0.03	-0.23	0.05	-0.08
GP61	0.25	0.17	0.06	0.34	0.18	0.13	0.22	0.17	0.09	0.14	0.22	0.17	-0.12	<b>0.69</b>	<b>0.41</b>	0.17	<b>0.57</b>	<b>0.34</b>	0.11	0.22	<b>0.19</b>	0.09	<b>0.75</b>	0.18	<b>0.61</b>
ALL	0.07	0.08	0.01	<b>0.14</b>	<b>0.08</b>	0.06	-0.00	0.06	0.04	0.06	0.05	0.04	0.03	0.04	0.04	0.05	<b>0.07</b>	<b>0.06</b>	<b>0.06</b>	<b>0.06</b>	0.04	0.06	0.07	0.03	0.1

Table 5. Pair-wise  $F_{ST}$  values for all sampling locations of the desert tortoise (below diagonal) and corresponding pair-wise geographic distances (km; above diagonal).

	RC	BD	MM	GB	MD	CS	NEL	NWL	AM	PA	SH	IV	WP	SI	SWL	SEL	EL	PI	CM	EP	CK	PM	OR	SC	FK
RC	-	56	94	90	129	131	158	186	260	242	279	250	307	226	209	181	208	238	312	310	439	399	398	384	434
BD	0.01	-	38	57	79	75	105	131	205	186	224	202	259	175	157	131	162	197	276	266	403	356	346	330	379
MM	0.01	0.01	-	55	48	41	68	93	170	147	186	168	225	140	121	98	131	169	251	235	377	325	310	292	341
GB	0.04	0.05	0.04	-	50	91	84	116	212	172	204	162	219	141	128	96	119	148	223	220	351	309	313	304	356
MD	0.03	0.05	0.04	0.04	-	61	34	66	166	122	155	123	180	97	81	52	83	121	203	188	329	278	270	257	309
CS	0.05	0.06	0.05	0.06	0.04	-	58	68	130	117	157	157	212	126	105	94	128	170	253	229	376	318	287	265	311
NEL	0.03	0.05	0.04	0.05	0.01	0.02	-	32	136	88	122	102	158	72	52	36	70	113	196	172	318	261	241	226	276
NWL	0.03	0.06	0.04	0.06	0.02	0.04	0.02	-	107	56	93	93	146	62	40	49	76	118	198	166	316	252	220	200	248
AM	0.05	0.07	0.07	0.08	0.04	0.05	0.03	0.04	-	78	102	168	198	144	130	154	173	209	278	232	377	302	226	186	217
PA	0.06	0.08	0.07	0.08	0.04	0.03	0.03	0.03	0.01	-	42	90	124	67	58	89	100	132	200	156	303	231	176	149	195
SH	0.05	0.08	0.07	0.1	0.04	0.05	0.03	0.04	0.04	0.02	-	84	97	75	78	113	111	131	185	134	276	200	135	108	155
IV	0.05	0.09	0.08	0.09	0.04	0.05	0.04	0.04	0.05	0.03	0.03	-	58	32	54	70	44	48	111	73	222	160	152	152	206
WP	0.08	0.12	0.09	0.11	0.05	0.06	0.05	0.05	0.05	0.04	0.03	0.02	-	87	108	128	100	83	95	40	180	108	102	116	169
SI	0.04	0.07	0.06	0.07	0.03	0.03	0.03	0.02	0.04	0.02	0.02	0.02	0.025	-	22	46	36	65	140	105	254	192	173	164	217
SWL	0.05	0.08	0.06	0.08	0.03	0.04	0.02	0.02	0.04	0.03	0.03	0.03	0.036	0.01	-	35	43	81	159	126	276	213	189	177	228
SEL	0.05	0.09	0.07	0.09	0.02	0.05	0.02	0.03	0.02	0.02	0.02	0.02	0.025	0.02	0.01	-	34	76	159	138	283	228	218	209	262
EL	0.06	0.09	0.08	0.1	0.05	0.06	0.05	0.04	0.04	0.04	0.03	0.02	0.019	0.02	0.03	0.01	-	43	125	105	248	195	196	194	248
PI	0.08	0.10	0.1	0.10	0.07	0.07	0.06	0.06	0.06	0.05	0.04	0.03	0.011	0.03	0.04	0.03	0.02	-	83	72	208	161	185	193	247
CM	0.11	0.15	0.14	0.14	0.10	0.11	0.1	0.09	0.09	0.08	0.07	0.06	0.033	0.07	0.07	0.06	0.05	0.02	-	55	127	100	176	204	253
EP	0.10	0.14	0.13	0.14	0.09	0.1	0.08	0.09	0.08	0.07	0.06	0.05	0.021	0.06	0.07	0.05	0.04	0.01	0.00	-	150	90	127	150	201
CK	0.12	0.16	0.14	0.15	0.11	0.12	0.10	0.11	0.12	0.10	0.08	0.08	0.046	0.08	0.08	0.07	0.06	0.03	0.04	0.03	-	81	197	243	274
PM	0.13	0.16	0.15	0.16	0.11	0.11	0.10	0.11	0.10	0.09	0.08	0.07	0.026	0.07	0.08	0.07	0.06	0.03	0.03	0.03	0.02	-	118	163	197
OR	0.12	0.15	0.15	0.15	0.1	0.11	0.1	0.10	0.09	0.09	0.07	0.07	0.026	0.07	0.08	0.06	0.05	0.03	0.04	0.03	0.04	0.01	-	49	81
SC	0.12	0.15	0.14	0.14	0.09	0.09	0.09	0.09	0.1	0.08	0.07	0.06	0.022	0.06	0.07	0.06	0.06	0.03	0.04	0.04	0.05	0.03	0.02	-	54
FK	0.12	0.16	0.14	0.15	0.09	0.10	0.09	0.10	0.09	0.08	0.08	0.07	0.032	0.07	0.07	0.06	0.06	0.05	0.06	0.05	0.05	0.03	0.03	0.01	-

## Supplemental Figures

Figures 1. Genotype clusters identified within the Northern Mojave cluster using hierarchical analyses in Program STRUCTURE. (a) Mean  $\ln(PD)$  and  $\Delta K$  and (b) representative bar plot for the Northern Mojave (NM) cluster, which split into two additional clusters.

Figure 2. Genotype clusters identified within the Las Vegas cluster using hierarchical analyses in Program STRUCTURE. (a) Mean  $\ln P(D)$  and  $\Delta K$  and (b) representative bar plot for the Las Vegas cluster, which splits into three additional clusters.

Figure 3. Genotype clusters identified within the California cluster using hierarchical analyses in Program STRUCTURE. (a) Mean  $\ln P(D)$  and  $\Delta K$  and (b) representative bar plot for the California cluster, which splits into four additional clusters.

Figure 1.

Figure 1a.

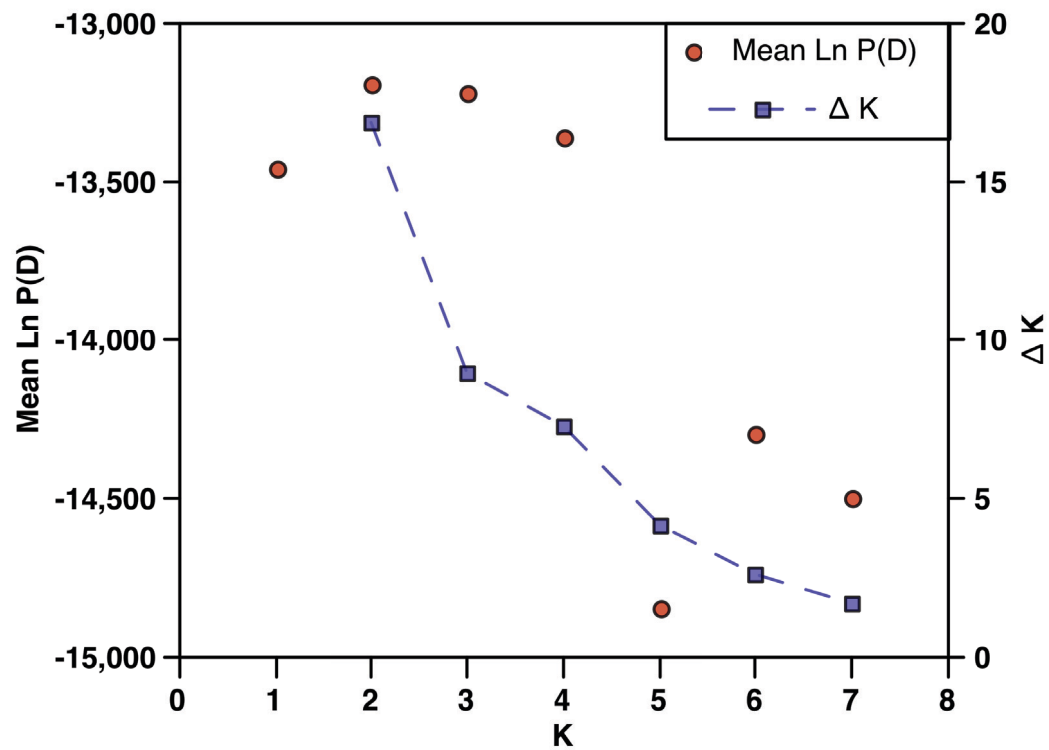


Figure 1b.

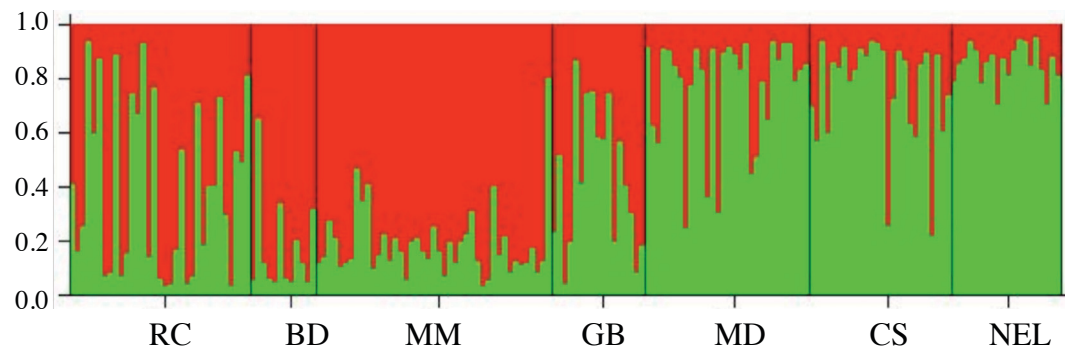


Figure 2.

Figure 2a.

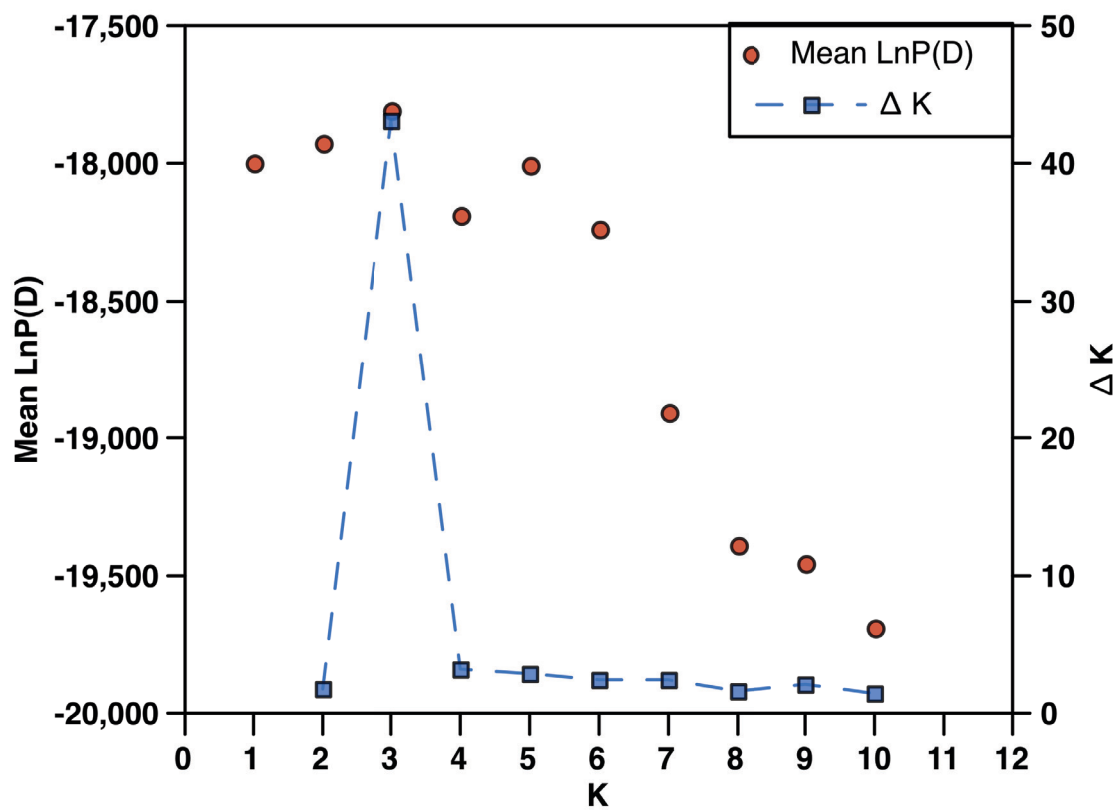


Figure 2b.

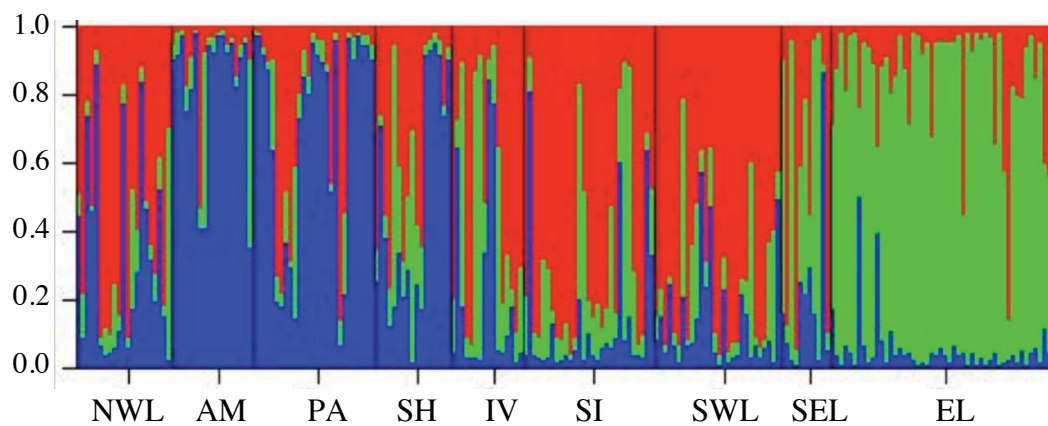


Figure 3.

Figure 3a.

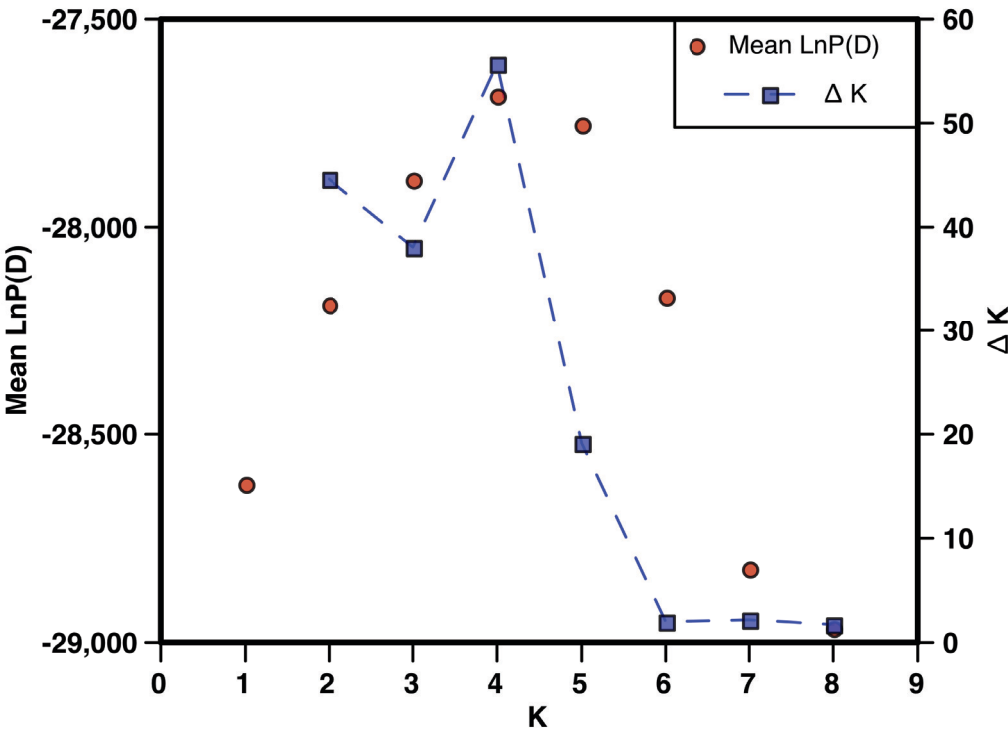


Figure 3b.

